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Seed Maturation in *Acacia auriculiformis*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

Department of Forest Science

EDMONTON, ALBERTA

Spring 1987

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Supervisor

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.....

Date January 12, 1987.

ABSTRACT

Seed maturation of *A. auriculiformis* A. Cunn. ex Benth. at Muak-Lek, Thailand in 1985 has been studied associated with germination, dormancy and seed coat development including some aspects on seed coat treatments. The seeds developed during the rainy season between June and October 1985. Seed began ripening by September 5 (82 days after anthesis). Brown pericarps and black seed coats could be used as a qualitative maturity index. Funiculus color was not a good indicator because of its fast development. Fruit and seed moisture content decreased with maturation. At maturity, fruit and seed moisture content were about 35% and 27% (fresh weight basis), respectively. Seed fresh weight and dry weight showed maturity when they remained at the constant level of 20-21 and 17 mg, respectively. Seed ripening was also investigated by X-ray radiographs, and seeds reached maturity after September 5. Loss of seed moisture was an important factor controlling germination and dormancy behavior during maturation. Germinability decreased while natural dormancy increased after seed moisture content fell below 23%. Furthermore, seeds showed imposed dormancy when they were harvested and dried rapidly before natural seed shedding took place. Seeds collected early tended to be more dormant than those collected late. The structure of immature seed coats differed from that of mature seed coats. The immature seed coats had a macrosclereid layer and parenchymatous layers while the mature seed coats had

differentiated more having three more layers; cuticle, hypodermal osteosclereids and inner osteosclereids. Seeds were morphologically fully mature when they showed compressed parenchyma cells, constant seed coat thickness on the flat sides of seeds and constant thickness of macrosclereid plus cuticular layer. Concentrated sulfuric acid, 95°C water and hand scarification treatments were able to make seed coats permeable to water in *A. auriculiformis*. Seeds soaking in 95°C water for 1 and 3 minutes gave the best total germination while hand scarification and concentrated sulfuric acid gave the best germination rate. Two months after collection, seed maturation still influenced the germination of seeds.

Key words: *Acacia auriculiformis*, seed maturation, germination, dormancy, seed coat structure and development, seed pretreatments.

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1. INTRODUCTION

1.1 Statement of the Problem

Large segments of the forest resources of tropical areas are being destroyed every year. Thailand is reported to lose 333,000 ha/year of its forest land base to other uses (Lanly 1982). In order to increase forest resources through expanding reforestation programs, large quantities of high quality tree seeds must be made available when required. Therefore, seed collection is of the highest priority in order to provide high quality seeds for nursery needs.

Foresters and seed collectors have long realized the need to collect seeds only when they are fully ripe, but before they are dispersed by fruit dehiscence or by dispersal agents. To achieve seed collection programs in order to collect high quality seeds, the collectors must be able to distinguish between ripe and unripe seeds or fruits and to know the stages and lengths of time for seed maturation. Seed collections which contain many immature seeds can bring about various problems for the nursery such as slow and incomplete germination, greater susceptibility to disease, reduced storage capability, increased incidence of abnormal seedlings and extraction of immature seeds being more difficult (Tanaka 1984).

Lack of technical knowledge of seed production and collection is among the main seed problems in tropical

countries. The ripening time of seeds of tropical species may vary greatly from year to year, from one location to the other and even from tree to tree within the same stand (Kittinanda 1975; Turnbull 1975). *A. auriculiformis* A. Cunn. ex Benth. is one of the tropical tree species which has this problem. For example, where this species grows in Muak-Lek, it begins to flower in May-June, but where it grows in Bangkok it usually flowers in January. Furthermore, the characteristic regeneration of *A. auriculiformis*, as well as many tropical species, is complicated by its sporadic flowering within the same tree. As a result, seed set of this species is not regular. In order to achieve seed collection objectives, the collectors need knowledge about seed maturation and development of this particular species.

Subsequently, methods by which germination will be maximally enhanced in the nursery must be developed. Generally, many researchers have recommended that the hard seeds of all *Acacia* species should be treated with manual scarification, boiling/hot water or concentrated sulfuric acid before sowing in the seed beds (Larsen 1964; ISTA 1976; Clemens et al. 1977; NAS 1979; Willan 1985). However, because of differences in seed coat thickness and other properties, it is necessary that each species be treated differently to get dormant seeds to germinate without damaging non-dormant seeds in the seed lots.

As a result, studies to investigate seed maturation and the development in *A. auriculiformis* will be helpful for the

understanding of germination and dormancy characteristics and the various aspects of seed pretreatments. With these, successful collection can be handled to get high quality seeds. Better seed quality and proper seed treatments are needed to produce vigorous seedlings for reforestation programs.

1.2 Purpose of the Study

The major aims of this study are:

1. To investigate the effectiveness of the various indices that indicate seed maturity in *A. auriculiformis*.

2. To examine the extent to which the seed coat anatomy and development of *A. auriculiformis* are related to its seed maturation, germination and dormancy.

3. To verify the safety and efficiency of the various pretreatment procedures for the dormant seeds in *A. auriculiformis* to be used in the nursery production of seedlings.

1.3 Background of Species

1.3.1 Life history of genus and species

The genus *Acacia* belongs to the family Leguminosae, subfamily Mimosoideae. Some authors have preferred Leguminosae (Fabaceae) in the order Leguminales, to be divided into three families; Mimosaceae, Caesalpiniaceae and Papilionaceae (Fabaceae) (Dnyansagar 1954; Gopal and

Thapliyal 1971; Hopper and Maslin 1978; Murray et al. 1978; Gunn 1981).

Acacia is a large tropical and subtropical genus occurring on all continents and Pacific Islands, except Europe and Antarctica (Atchinson 1948). While the exact number of species is unknown, recently the genus *Acacia* consisted of about 1,200 species, chiefly in Australia and Africa, with about 700 of these species being endemic to Australia (Guinet and Vassal 1978).

Acacia was first classified into six series: Filicinae, Vulgares, Botryocephalae, Phyllodineae, Pulchellae and Gummiferae (Bentham 1875, cited by Guinet and Vassal 1978 and Doran et al. 1983). Recently *Acacia* was subdivided by Guinet and Vassal (1978) into three groups taking into account new characters of pollen, seeds and seedlings. The classification is as follows:

Group I : Subgenus Aculeiferum Vassal
(= Bentham's series Filicinae and Vulgares)

Group II : Subgenus Heterophyllum Vassal
(= Bentham's series Botryocephalae, Phylloidineae and Pulchellae)

Group III : Subgenus Acacia Vassal
(= Bentham's series Gummiferae).

These three subgenera have been subdivided into sections by Vassal (1972, cited by Pettigrew and Watson 1975 and Guinet and Vassal 1978) as follows:

Subgenus I : Section Aculeiferum
Section Monacanthea
Section Filicinum
Subgenus II : Section Heterophyllum
Section Uninervea
Section Pulchelloideae
Subgenus III : Section Acacia

In brief, the main difference among those subgenera is that Heterophyllum is bipinnate or phylloidinous species while Aculeiferum and Acacia are only bipinnate. For more information on comparisons among subgenera and sections see Guinet and Vassal (1978), and for Australian *Acacia* species see Tindale and Roux (1969, 1974); Pettigrew and Watson (1975); and Hopper and Maslin (1978). All Australian *Acacias* have been included in subgenus Heterophyllum.

Atchison (1948) divided *Acacias* by chromosome numbers into three geographical groups of species : Australian and Pacific Islands species (2n = 26), American and West Indian species (2n = 26) and Asiatic and African species (2n = 52, 104, 208).

Acacia auriculiformis (formerly spelled *A. auriculaeformis*) is a phylloidinous Australian species in section Heterophyllum, corresponding to section Juliflorae of Bentham's subseries (Tindale and Roux 1969; Pettigrew and Watson 1975). The species is native to savannas of Papua New Guinea, to the islands of Torres Strait and to the northern part of Australia (Corner 1952; NAS 1979,1980). This species

has now been introduced into Southeast Asian countries, India, Tanzania and Nigeria. This species is an exotic for Thailand. It was introduced into Thailand as an ornamental plant from Australia by lieutenant Khun-narongchawanakit in 1935 (Pinyopusarerk 1984). Because of its beautiful flowers and its ability to grow in many locations, it has been distributed throughout the country.

1.3.2 External descriptions of species

A. auriculiformis produces simple indeterminate inflorescences termed spikes which develop in centrifugal direction. The spikes are axillary and the axillary spikes are usually solitary or paired, sometimes in three spikes. The flowers are small and yellow. The floral formula is K(5), C(5), Aα, G1 (Dnyansagar 1958). The fruits of *A. auriculiformis* are dehiscent along the sutures of pericarp termed 'legumes or pods' (Esau 1977). Gopal and Thapliyal (1971) who identified Mimosoid seeds particularly by the hilum, the shapes and the sizes of seeds and the sizes of 'rings' (so called 'pleurograms' by Corner 1951) on the surfaces of seeds gave descriptions of *A. auriculiformis* seeds as follows:

> "Seed elliptic or orbicular; ring visible only under magnification of the order X10 as a fine 'U' shaped line. Seed flattened; homogeneously olive brown, dark brown, nut brown or chocolate, smooth or shining; 4-6 mm long, 3.5-5 mm wide; hilum situated in a shallow groove."

Little is known about this species and whether fruits and seeds are very different in condition when they are collected. However, Buttrose et al. (1981) illustrated that South Australia inflorescences of *Acacia pycnantha* exhibit synchronous development. *A. pycnantha* flowers which are produced every month of the year show differences in the rate of development. Late flowers develop more rapidly than early flowers; as a result, they reach anthesis in the same month.

1.4 Literature Review

1.4.1 Seed maturation

Many investigators have stated that seed maturity can be indicated in terms of physical, biochemical, morphological, physiological and climatic indices (Harrington 1972; Turnbull 1975; Edwards 1980; Tanaka 1984; Willan 1985).

Physical indicators

Physical maturity indices are the most practical for collectors unless the collection areas happen to be close to the laboratory as with some seed orchards. This type of indicator is based on the physical parameters of the fruits and seeds in a number of ways, for example, by color differences, by moisture content and specific gravity, and by changes in size.

a. Fruit color. Color changes in fruits provide a simple and, in some species, reliable criterion for judging seed maturity. With these the collectors need experience in the characteristics of the species concerned. Color changes may be accompanied by hardening or dehiscence of the pericarp of woody fruits (Barner and Oleson 1984; Willan 1985). Color changes were found to be the most reliable indicator of maturity for some economic species in the Central Mississippi without any other obvious physical or chemical changes indicating maturity (Bonner 1972, 1975, 1976a). Bonner (1976b) also showed the differences of fruit color changes between *Quercus shumardii* Buckl. and *Q. alba* L. acorns. At maturity *Q. alba* acorns are light brown, and *Q. shumardii* acorns are dark reddish brown. Commercial harvests of *Coronilla varia* L. occur when a majority of the seed pods are either dull or reddish-brown (Peiffer et al. 1972). Pericarps of *Tilia americana* L. also show maturity by turning from green to greyish-brown (Vanstone and Ronald 1982). In Malaysia, *Acacia mangium* Willd. was reported ready for harvest when the majority of pods in the crown were dark brown to black in color and associated with the cracking of the dark pods (Bowen 1981; Bowen and Eusebio 1981).

b. Seed color. In general, seed color is not a good criterion for defining maturity. However, seed-wing color has been recommended as a good index. The development of brown coloration in the seed wing is a promising qualitative indicator for *Shorea siamensis* Mig. (Panochit et al. 1984). The development of orange funicles of *A. mangium* seeds

showed that they are able to germinate (Bowen and Eusebio 1981).

c. Specific gravity of fruits. Specific gravity is the ratio of unit weight to unit volume. It is the most popular test of maturation in the field in the temperate zone, especially for coniferous cones (Tanaka 1984; Willan 1985). The decrease in specific gravity of cones appears to be closely associated with seed ripening. However, the specific gravity of each species, first, must be known by placing mature fruits or cones in various liquids with known specific gravity such as water (SG=1.0), lineseed oil (SG=0.93), light motor oil (SG=0.88), various mixture of kerosene (SG=0.80) and so on. For example, if they correspond to water; thus, their specific gravity is equal to 1.0. Then, water is used to test seed maturity of that species. If seeds are mature, fruits or cones will float and they sink if immature.

d. Fruit and seed moisture content. Moisture content plays the most important role in seed development and maturation. Water loss of maturing fruits and seeds is closely related to the maturity of seeds. When approaching seed maturity, pod moisture content was between 30% and 50% for *Acacia catechu* Willd. (Amata-archachai and Hellum 1984). Panochit et al. (1984) indicated that the critical minimal moisture content for collection of *Shorea siamensis* lay between 77.71% and 91.99% for fruits and between 87.1% and 102.32% for seeds because this seed type belongs to the

recalcitrant seed type.[1] Some authors indicated that because of very gradual decreases in moisture content of fruits and seeds, and because of variation in local weather conditions, moisture content is not a good indicator of seed maturity for some species (Bonner 1976a; Liengsiri and Hellum 1984).

e. Fruit and seed size. Measurement of seed size (diameter) is far less important when assessing seed ripeness as forest tree fruits generally achieve near full size relatively early in seed development so that changes in physical dimension at full maturity usually are too small to indicate maturity (Edwards 1980). Bonner (1972, 1975, 1976b) showed that fruit and seed size failed to indicate seed ripeness. However, Liengsiri and Hellum (1984) showed that the cone growth of *Casuarina equisetifolia* L. was more rapid in diameter than in length and both cone growth in diameter and in length exhibited a typical sigmoid growth pattern over the period of 140 days to reach full size. They suggested that fully developed cones may even shrink slightly as they began to dry out during late maturation.

Biochemical indicators

Biochemical changes take place within ripening seeds and little is known for most species. Changes in biochemical concentration during maturation have been investigated with regard to differences in crude fat oil, soluble nitrogen, protein- nitrogen, starch (soluble and insoluble

---

[1] Recalcitrant seed: seed which has short longevity and can not be stored well with low moisture content (Bewley and Black 1982).

carbohydrate), and phosphorus, magnesium and calcium concentration. Leininger and Urie (1964) determined that the percent oil content of *Cartbamus tinctorius* L. seeds increased gradually to a maximum approximately 28 days after flowering. Bonner (1972) reported on data on chemical content of seeds of *Liquidambar styraciflua* L. and *Platanus occidentalis* L. He measured increases in crude fat and protein-nitrogen, and decreases in soluble nitrogen and soluble carbohydrate, and little changes in phosphorus, magnesium and calcium concentration as seeds ripening. Similar results were shown in *Prunus serotina* Ehrh. fruits (Bonner 1975), in *Liriodendron tulipifera* L. seeds (Bonner 1976a) and in *Shorea robusta* (Nautiyal and Purohit 1985). Hellum et al. (1983) showed that the chlorophyll content (analyzed by a scanning spectrophotometer at 662 nm.) in cones of *Pinus contorta* Dougl. var *latifolia* Engelm. decreased as cones ripened. A similar result was shown by Nautiyal and Purohit (1985) that the total chlorophyll as well as carotenoids of *Shorea robusta* seeds decreased for 35 days following anthesis. The chlorophyll a:b ratio also increased progressively during 35 days after anthesis indicating a faster rate of degradation of chlorophyll-b than chlorophyll a; thereafter, it continually decreased with time probably because the embryo switched over from its heterotropic nature to autotropic mode.

Abscisic acid (ABA) concentration in the seeds influences seed maturity as shown in the study in developing grains of *Triticum aestivum* L. (King 1976). Early in

development, the content of ABA per wheat grain is quite low. Subsequently, it increases parallel to dry matter accumulation. When the grain starts to dry, the ABA concentration decreases. Thus King suggested that it is possible that drying of the grain enhances ABA destruction, which may possibly induce grain maturation.

Morphological indicators

Morphologically mature seeds have fully developed embryos, endosperm and other structures (Edwards 1980). Ripeness generally is described as an index of embryo development for many species, particularly conifers. Preliminary examination of seed content can be done in the field by observing the texture or firmness of the embryo, endosperm and seed coat. Turnbull (1975) pointed out that seeds of many species can be collected when most embryo and endosperm pass through a 'dough' stage as tissues appear more firm.

Ching and Ching (1962) used the relationship between embryo and embryo cavity as a possible index of *Pseudotsuga menziesii* Mirb. cone maturation: cones should be collected when the length of embryo is 90% or more of the length of the embryo cavity in seed located in the middle portion of the cone. Also a relative embryo length of 75% is widely accepted in British Columbia as the point at which cone collection in most conifer species can begin (Edwards 1980). Greenwood and Bewley (1981) constructed a morphological timetable of the development of *Ricinus communis* L. cv. Hale

seeds. They divided growth into 10 stages from proembryo through maturation to quiescence taking approximately 60 days under the growth condition used.

Besides embryo and endosperm examination, the testa also has been used to indicate maturity. The thickness of the testa of *Tilia americana* did not change during maturation (Vanstone and Ronald 1982); however, the testa showed lignification and impregnation with cellulose as seeds matured. Spurny (1964) studied the development of suberin formation on the apical part of the cell walls of macrosclereid in the seed coat of *Pisum sativum* L. in the course of seed ripening. Suberin formation began to develop about 14 days prior to full seed maturity, and fully developed suberin was found as late as in the seed coat of fully mature seeds.

The technique of X-ray radiographs has been widely used as a quick and relatively reliable method to examine the development of the embryo and endosperm (Swaminathan and Kamra 1961; Turnbull 1975). Hellum et al. (1983) used X-ray radiographs to evaluate the stages of maturation by looking at embryo and endosperm in *Pinus contorta* seeds.

During seed maturation, morphological and anatomical structures of seed, particularly of seed coat of orthodox seeds[2], increase in degree of hardness and impermeability to water, to oxygen and resistance to radicle protrusion. Anatomical structures associated with seed dormancy are reviewed later.

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[2]Orthodox seed: seed which can be stored with low moisture content (Bewley and Black 1982).

Physiological indicators

During morphological development nutrient reserves flow from mother plant into the growing parts of the seed. When this flow stops, the seed should survive if detached. At this point organic accumulation within the seed has been completed, as shown by no further increase in dry weight, and seeds are said to have reached their physiological maturity (Harrington 1972; Edwards 1980). Many researchers have used dry weight and/or fresh weight to demonstrate seed maturation (Ching and Ching 1962; Leininger and Urie 1964; Pfister 1967; Rediske 1969; Bonner 1972, 1976a, 1976b; Vanstone and Ronald 1982; Amata-archachai and Hellum 1984; Maiti et al. 1985).

Not only seed dry weight and fresh weight but also germination behavior, particularly concerned with seedling productions, is a good indicator of maturity (Allan 1958a, 1958b; Bonner 1972, 1976a; Vanstone and Ronald 1982). Allan (1958a) showed that germination capacity indicated mature stage of *Tsuga heterophylla* (Rafn.) Sarg. seeds. In addition, the rate of germination increased as total germination increased for increasingly later collections. Furthermore, although *Tsuga heterophylla* seed were stratified and stored for two years, the date of collection still influenced rate of germination. Similar results were found in *Pseudotsuga menziesii* (Allan 1958b), but not in *Abies grandis* (Pfister 1967). Germination rate of *Abies grandis* was not significantly different between early and late collections. However, maximal total germination of

Abies grandis was given at maturity.

As reviewed above, seed maturation for any species commonly can not be defined by using only one indicator. To determine the stage when seeds enter maturity, it is necessary to study many indicators and try to find which one or which combination can best define maturity.

1.4.2 Seed coat structure and development

The processes of seed development and maturation begin at the moment of fertilization. Thereafter, the ovule becomes the seed. Seeds of angiosperms are composed of an embryo, endosperm and seed coat (Fahn 1967; Esau 1977). The integuments of the ovule undergo conspicuous changes before becoming the seed coat. The degree to which integumentary tissues contribute to form the seed coat is extremely varied depending on species. Among families or genera there are different and similar cellular structures as well as seed coat development. For example, Columiferae shows a close connection with Euphorbiaceae and Thymelaeaceae in the development of the integuments, and Centrospermae and Plumbaginaceae form a group of families with similar development of the integuments (Wunderlich 1967).

Foster and Gifford (1974) recommended that there should be four cases to consider in the seed coat development of angiosperm seeds: (i) differences in degree of destruction of portions of the integuments during ontogeny, (ii) the presence of certain cell types in seed coat during ontogeny, (iii) the pattern of the vascular system, and (iv) the

formation of specialized cells.

Many investigators reported that the ovule of Mimosoideae is composed of two integuments: an outer and an inner integument (Davis 1966; Fahn 1967; Bhatnagar and Johri 1972). The seed coat is formed only by the outer integument alone while the inner one and the nucellus are crushed or obliterated by an enlargement of the embryo sac (Fahn 1967; Bhatnagar and Johri 1972; Corner 1976). However, the inner integument still persists even in the mature seed of *Calliandra hematocephala* Hassk., a member of Mimosoideae (Dnyansagar 1958).

Corner (1951) studied seed anatomy in 71 species of Leguminosae. He figured that the general characteristic features of Leguminous seed coat are the presence of some layers of cuticle, macrosclereid cells (also called 'Malpighian cells' or 'Palisade cells'), osteosclereid cells (also called 'Hour-glass cells'), and parenchyma cells (also called 'Mesophyll' or 'Nutrient cells'). Bukovac et al. (1981) defined plant cuticle as a nonliving, noncellular lipoidal membrane covering on the outer surface with embedded wax. The development of the cuticular layer in the seed coat is derived from the integument, the nucellar epidermis and part of the inner pericarp (Esau 1977).

Macrosclereids are elongated and thick-walled cells. The chief constituents of macrosclereid cell walls are cellulose and pectic substances (Hamly 1932; Werker et al. 1979). The fibrillar structures between primary and secondary walls of macrosclereids show no distinctions in

Pisum spp. (Reeve 1946a). The secondary wall thickening of macrosclereids also shows a strong birefrigence with polarizing microscope (Reeve 1946a). Macrosclereids of some species can be segregated into two parts with the separation of the 'Light line': the lower part is the body of the cells and the upper one is so called 'Cap' by Hamly (1932). Reeve (1946a) and Scott et al. (1962) explained that the light line is a phenomenon of light refraction which is caused by changing of microfibrillar orientation between the body and the cap. The microfibrillar orientation of the body is parallel with the length of the cell while the cap shows a characteristic of transverse microfibrillar striation. However, there is no light line in some leguminous seeds (Corner 1951; Spurny 1972; Swanson et al. 1985). The thickening walls of the upper part are fluted and twisted at maturity (Reeve 1946a; Spurny 1972). Spurny (1972) indicated wall thickening of macrosclereids in Leguminosae that genus *Tetragonolobus* showed the noncellulosic walls while genus *Medico, Melilotus* and *Trifolium* (except *Trifolium incarnatum*) presented in form of the body and the cap.

Osteosclereids are columnar cells with thickened longitudinal walls which expand on both ends of the cells. The development of the osteosclereid cell of *Pisum sativum* was investigated by Reeve (1946b) and Harris (1984). The osteosclereids mature after the macrosclereids. The osteosclereid precursors are small, densely protoplasmic cells. Eventually, the walls in the center portion of the cells become heavily thickened preventing future expansion

while the ends of the cells are still less thickening and continue to expand creating the typical bone-shaped cells. The cells are separated from each other with the large interconnecting intercellular spaces. The cellulosic microfibrillar orientation of osteosclereid walls is parallel with the long axis of the cell similar to those of the body cell walls of macrosclereid cells (Reeve 1946a). Usually these cells occupy a layer under the macrosclereids in Leguminosae (Corner 1951, 1976).

The parenchyma cells form the main body of the seed coat of leguminous seeds. In some cases the cells compress when seeds mature such as in genus *Pisum* (Werker et al. 1979), in *Trifolium subterraneum* (Aitken 1939), and in *Coronilla varia* L. (Mckee et al. 1977). Also the parenchyma cells exhibit thick walls in *Vicia faba* L. (McEwen et al. 1974), and in *Glycine max* (Throne 1981).

Corner (1976) classified the seeds of dicotyledons according to a mechanical layer (macrosclereid or fibrous layer) in seed coats. He stated that leguminous seeds are included into the group called 'exotestal seeds' which have the macrosclereid cells as the mechanical layer differentiated from the outermost cells of the outer integument.

The patterns of integumentary venations of ovules are extremely diversified. Corner (1976) stated that the vascular bundle of Mimosoideae extends as a curved and unbranched vein from hilum through chalaza to the micropylar end of the outer integument.

1.4.3 Seed dormancy and germination

From an ecological point of view, seed dormancy is a part of the regenerative strategy in the life cycle of many flowering plants (Harper 1977; Grime 1983). When flowering plants produce their seeds, their populations are able to expand with the process of seed dispersal into the seed bank. With respect to the fate of their seeds, two contrasting groups may be recognized. In one, most but not all of the seeds germinate soon after falling to the ground while in the other, many seeds become persistent in the seed bank as dormant seeds which wait to overcome their dormant stage (William and Elliott 1960; Harper 1977; Grime 1983). Obviously, the former lacks any persistent seed dormancy. This may explain why the species does not persist on dry sites because moisture conditions do not allow for seedling establishment after germination. The latter is advantageous in the way that the dormant seeds do not germinate if the germination conditions are suitable, and they still remain viable. The existence and duration of a dormant phase are synchronized with the environmental and inherited conditions of the seeds (Harper 1977; Bewley and Black 1982). The evolutionary experiences of species lead them to end their seed dormancy when they have the best chance of survival (Harper 1977). In nature, seed dormancy may terminate by fire (Stone and Juhren 1951; Rolston 1978), by alternate wet and dry cycle, by high temperatures, by rainfall and soil moisture (Aitken 1939; William and Elliott 1960; Longman 1969; Seif El Din and Obeid 1971), by soil microflora

(Aitken 1939), by digestion system of animals or birds (Rolston 1978) and other similar means. Dexter (1955) concluded that (i) hard contents of seeds in *Medicago spp.* lots from lower elevation were lower than those from high elevation, (ii) seeds harvested late in cool weather appeared to have higher hard seed contents than those harvested in warmer weather earlier in the season, and (iii) seeds from irrigated areas had less hard seed contents than those from humid areas.

In the tropics, the ecological significance of seed dormancy appears to be more closely related to survival during dry period and seedling establishment during the rainy season (Longman 1969). With a distinct dry season, germination typically occurs when the rains come and soil moisture is high. The frequency and intensity of rainfall may decide the germination and survival of seed during the rainy season. Seif El Din and Obeid (1971) concluded that the peak germination of *Acacia senegal* (L.) Willd. seeds in the field was reached two days after a single heavy rain.

Generally, seed dormancy indicates the inability of seed to germinate under favorable conditions. This inability to germination is due to any number environmental conditions. Seed dormancy can be classified into several different types. For example, Harper (1977) classified seed dormancy into three categories so called innate, induced and enforced dormancy. Nikolaeva (1969) divided dormancy into simplified forms: exogenous or seed coat dormancy, endogenous or embryo dormancy and combined

exogenous/endogenous dormancy. Bewley and Black (1982) preferred to use the terms primary dormancy, relative dormancy and secondary dormancy. However, as a whole, in this thesis seed dormancy is reviewed in the light of seed coat dormancy (corresponding to innate dormancy and primary dormancy).

1.4.3.1 Seed coat structure and impermeability

It is interesting to note that the structures of the seed coat are functional for the impermeability to water, impermeability to oxygen and as a mechanical barrier to radicle protrusion (Barton 1947; Ballard 1973; Rolston 1978; Werker 1980/81). The greatest numbers of species with hard seeds belong to the families Cannaceae, Chenopodiaceae, Convallariaceae, Convolvulaceae, Geraniaceae, Gramineae, Leguminosae, Liliaceae and Malvaceae (Werker 1980/81). The structures and functions of the seed coats of Leguminosae have been investigated in many species (Hamly 1932; Reeve 1946a, 1946b; Watson 1948; Corner 1951, 1976; Hyde 1954; Hagon and Ballard 1970; Ballard 1976; Werker et al. 1979).

As already mentioned by Corner (1951), the major cell types of the seed coat of leguminous seeds are macrosclereids (covered with the cuticle on the seed coat surface), osteosclereids, and parenchyma cells. There are some conflicts in the literature whether cuticle is permeable to water. Bukovac et al. (1981) indicated that the epicuticular wax of the cuticle is the prime barrier to penetration of water and chemicals. White (1908) concluded

that the impermeability of the seed coat of many species was due to the presence of the developed cuticle during ripening. Similarly, the seed coat of *Acacia melanoxylon*, *Cytisus albus*, *Indigofera arrecta* (Rees 1911) and *Prosopis stephaniana* (Khudairi 1956) are impermeable to water because of the presence of the thick cuticle. Contrary to those results, Ballard (1973) showed that it is not the waxy cuticular layer which is playing a role in impermeability to water because (i) solvent removal does not make the seed any more permeable, (ii) cutin stains with aqueous dyes, and (iii) water can be transported through the cuticle to remote punctures in the testa. Also Watson (1948) examined 21 species in Papilionaceae and pointed out that the variations in the cuticular thickness were so small that they were very difficult to record and could not be linked with the degree of impermeability of a seed. Swanson et al. (1985) also indicated that a waxy cuticular layer of the *Pheaseols vulgaris* seed coat is not ready evidence of impermeability because the wax may have been removed by ethanol. Sharma et al. (1977) illustrated that two major types of seed coat pattern of *Phaseolus spp.* have been recognized: (i) the heavy and papillate wax deposition on seed coats which are associated with the dormancy of seeds in wild populations, and (ii) the scanty and smooth wax deposition which correlates with lack of dormancy in the cultivated seeds.

Many investigators have indicated that the significant functions of the macrosclereid layer is to create hardness of seed coats and impermeability to water. Watson (1948)

indicated that the suberized and cutinized subcuticular layers of macrosclereid cells were the main barrier to seed coat permeability. Corner (1951) pointed out that hardseededness and impermeability of testa in Leguminosae are caused mainly by the contraction of the walls of macrosclereid cells as they ripen. Reeve (1946b) also stated that macrosclereid cells shrink in length without any change in diameter when the tissues dry during seed ripening, and the shrinkage results in a 10% to 20% decrease in length. In the genus *Pisum*, the pectinaceous layers of the caps and the presence of quinones in the macrosclereids are responsible for seed coat impermeability to water (Werker et al. 1979).

No evidence supports a major role of osteosclereid cells in impermeability of the seed coat. However, the layer of osteosclereids showed lengths nearly double that of macrosclereids in *Glycine max* (L.) Merr. seed (Throne 1981).

Parenchyma cells in the seed coat show typical water permeability (Hamly 1932; Aitken 1939). According to Aitken (1939), these cells in the seed coat of *Trifolium subterraneum* L. expanded up to about three times their natural thickness when seeds absorbed water while macrosclereids expanded only 10% of their thickness.

1.4.3.2 Overcoming seed dormancy and permeability

As far as we know the physical properties of the seed coat are involved in seed dormancy. The seed coat functions in control of water uptake. Softening of seed coats allows water entry thus changing the seed from the dormant to the

non-dormant state. Numerous treatments have been experimented on to break dormancy, largely under laboratory conditions. Some of these are chemical treatments unlikely to occur in nature. However, they can be used so much better to terminate dormancy. Treatments that are generally effective in improving germination of hard seeds include:

a. Hand and mechanical scarification. This is the simplest, most reliable and most direct physical method (Willan 1985). This can be done by chipping, piercing, milling, rubbing or filing at the cotyledon ends of the seed. ISTA (1981) has recommended that piercing with a needle gives the highest germination with fewer abnormal seedlings than other methods. ISTA has also stated that scarification should take place on the shoulder of the seed one quarter of the way round the circumference from the micropyle. Scarification elsewhere can lead to damage to the embryo. Similar to ISTA's recommendation, Ponoy et al. (1984) recommended for *Leucaena leucocephala* (Lam.) de Wit. seeds that the best germination and seedling performances can be obtained by dissecting or clipping the testa at the cotyledon ends less than one quarter the length of the seed. Hsiao et al. (1983) also found that cutting the dormant caryopsis of *Avena fatua* L. transversely at half the distance from the end and placing the seeds vertically resulted in more than 90% germination within 36 hours.

Bebawi and Mohamed (1985) studied the germination response of six Sudanese *Acacias* to different seed coat scarification treatments. Seed scarification was made by

rubbing the seed coat until internal structures appeared. These seeds were rubbed in three positions: (i) at the micropylar end, (ii) at the chalazal end, and (iii) at both micropylar and chalazal ends. They found that rubbing scarification at any one location on the seed coat of *Acacias* would induce an almost equal effect on germination response. These scarification methods promoted germination on average 40.7% above the control.

According to the result from Brant et al. (1971), seeds of *Coronilla varia* L. scarified with a needle completely eliminated hard seeds to zero and increased total germination to 95%. They also suggested that mechanical scarification resulted in fissures penetrating the cuticle and macrosclereid layer. Piercing on *Avena fatua* caryopsis also showed that the needle penetrated the pericarp, the aleurone layer and the peripheral layers of the endosperm (Hsiao et al. 1983). Hsiao et al. (1983) also showed the distance effect of piercing on the germination response. Each 1 mm increase in distance from the embryo, the rate of germination was significantly reduced. Duran and Tortosa (1985) indicated that mechanical scarification of *Sinapis arvensis* L. seeds significantly increased germination and germination rate due to elimination of mucilage layer of the seed coat.

Clemens et al. (1977) concluded that manual scarification of seeds of five Australian *Acacias* gave large improvement in germination rate. Seeds completed germination within 8 days. However, they stated that this method can not

be guaranteed to promote germination of all viable seeds as some lots of *Acacias* seeds gave low germination percentage.

b. Boiling and hot water. These treatments have given good results with a number of leguminous seeds. The precise conditions of water temperature and soaking time have to be determined by experiments for each species. Factors such as the ratio of the volume of water to the volume of seeds may affect the rate of heating. It may vary considerably according to species, for example, ratio of 5-10 times for *Acacia mangium* (Bowen 1981; Bowen and Eusebio 1981). After the seeds are placed in boiling or hot water this treatment is usually followed by leaving the seeds in cold water for some time to allow water entry into the seed before sowing.

An initial temperature of 100°C gave good result in germination of *Prosopis stephaniana* (Khudairi 1956), *Acacia falcata* Willd. var *longifolia* (Clemens et al. 1977), *Coronilla varia* (Brant et al. 1971), *Gymnocladus dioicus* (L.) C. Koch, *Gleditsia triacanthos* var *inermis* L. and *Cercis canadensis* L. (Liu et al. 1981) and *Acacia mangium* (Bowen 1981; Bowen and Eusebio 1981). Some species respond better to an initial temperature below boiling point, for example, an initial temperature of 80°C for *Acacia terminalis* (Salisb.) Macbride and *Acacia suaveolens* (Sm.) Willd. (Clemens et al. 1977), 80-90°C for *Ceanothus sanguineus* Fursh. (Gratkoski 1962), and 85°C for *Cassia siamea* Britt. (Kobmoo and Hellum 1984). Both temperature and time influence outcome of hot water tests. For instance, seed germination of *Cassia siamea* at 95°C for one minute

reached 77.5% while it reached 82-83% at 85°C for 8-12 minutes (Kobmoo and Hellum 1984).

Boiling and hot water causes the seed coat to change in its properties. The seed coat of three woody legume species (*Gymnocladus dioicus*, *Gleditsia triacanthos* and *Cercis canadensis*) showed cracked surfaces, loss of waxy substance and rupturing of macrosclereid layer after placing in boiling water for a minute (Liu et al. 1981). Similar results were obtained with *Coronilla varia*; the columns of macrosclereid cells were separated after boiling the seeds (Brant et al. 1971). Christiansen and Moore (1959) also reported that hot water treatment of cotton seeds caused disruption of the seal at the chalazal region.

c. Chemical treatments. Concentrated sulfuric acid (H_2SO_4) is the most commonly used chemical to break dormancy in hard seeds. Seeds which are scarified with acid should be washed thoroughly for about 10 minutes in running water afterward to ensure that all the seeds are free of acid (Kemp 1975). Acid treatments are more effective than boiling or hot water treatment in some species, for example, in *Gymnocladus dioicus*, *Gleditsia triacanthos* and *Cercis canadensis* (Liu et al. 1981), in *Coronilla varia* (Brant et al. 1971), in *Prosopis stephaniana* (Khudairi 1956) and in *Cassia siamea* (Kobmoo and Hellum 1984). However, the length of time required for acid treatment has to be determined for each species. The treatment period that yields a high percentage of germination without visible injury is the right one to use. Overimmersing may injure the seed and

damage the embryo while insufficient immersing leaves the seeds still impermeable. Kemp (1975) stated that soaking time required for acid treatment varies not only between species but also between provenances and even between seed lots of the same provenance.

The effect of acid on seed coat structures has been shown by many investigators. Duran and Tortosa (1985) stated that the main action of concentrated sulfuric acid on *Sinapis arvensis* L. seed coat is disorganization of the structure of mucilage presented over the epidermis cells. Their results showed that seeds immersed in concentrated H_2SO_4 for four minutes gave the maximal germination of 79%.

Seeds of *Lupinus angustifolius* after acid scarification revealed that the overlying parenchyma and palisade cells at the hilar region were hydrolyzed while palisade layer on the remaining part of seed coat was disorganized (Burns 1959). Therefore, he concluded that entry of water occurs through the hilar fissure or pits eroded through the seed coat. Acid treatment also destroyed the areas surrounding both the micropyle and the hilum of seeds of *Rhus ovata* (Stone and Juhren 1951). It also dissolved the caps of macrosclereid cells and exposed the lumens of these cells in legume seeds (Brant et al. 1971; Liu et al. 1981).

ISTA (1976) recommended that breaking dormancy in *Acacia* species should be done by immersing seeds for one hour in concentrated sulfuric acid. Then seeds are washed thoroughly in running water after acid scarification and before sowing.

Duran and Tortosa (1985) experimented using potassium hydroxide (KOH) as a treatment to break dormancy in *Sinapis arvensis* L. seeds. Their result showed that potassium hydroxide was a moderate treatment because seeds soaked in KOH (10N) for 6 minutes gave the maximal germination of 67%.

Besides the previous treatments, liquid nitrogen has been used to break dormancy by reducing hard seeds of *Coronilla varia* to 15% and increasing germination to 65% (Brant et al. 1971). In contrast, it resulted in poor germination in *Gymnocladus dioicus*, *Gleditsia triacanthos* and *Cercis canadensis* (Liu et al. 1981).

Tran (1979) used microwave energy at 2450 MHz to break dormancy in Australian *Acacias*: *A. longifolia* and *A. sophorae*. It showed that microwave energy was as effective as boiling water, but it took one week longer for germination to reach 80% than for seeds treated with boiling water. He stated that microwave energy caused the strophiole[3] to appear golden and well defined under the microscope and to become permeable to water.

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[3]Strophiole : a small, raised area seen on the surface of seed coat close to the hilum but on the side opposite the micropyle or on the raphe between the hilum and the chalaza, typical to the Papilionoideae (Ballard 1976; Canavagh 1980; Werker 1980/81).

2. MATERIALS AND METHODS

2.1 Seed Collection Area

Fruits and seeds used for this study were collected in 1985 from the school yard of the Muak-Lek High School, Muak-Lek, Saraburi province, Thailand, about 1 km from the ASEAN-Canada Forest Tree Seed Centre. This centre lies at latitude N 14° 40' and longitude E 101° 17', at about 200 m altitude (Figure 1).

The seed collection area was located in the area between the 'Central Highland' and the 'Central Plain' (Figure 1). The central highland is typified by its undulating to rolling peneplains interspersed with step hilly areas and some very steep limestone escarpments (Gliessman 1983). The parent materials are limestone and shale which contain Fe-oxide 8-10%. The top soil color is dark brown to yellowish brown. Soil types are sandy loam, clay loam and clay (Gliessman 1983).

Climatic data of the seed collection area were obtained from the Thai-Danish Diary Farm which is about 500 m from the seed collection area. The ecological climatic diagram was constructed from 6-year data (1974-1979) following Walter's (1979) climate diagram (Figure 2). The typical climate of Thailand is the monsoon type. The humid season of this seed collection area ranges from April-May to October-November. The mean temperature throughout the year is in the range from 23°C in December to 29°C in April.

Figure 1. Map showing seed collection area and the main physiographic regions of Thailand (after Gliessman 1983).

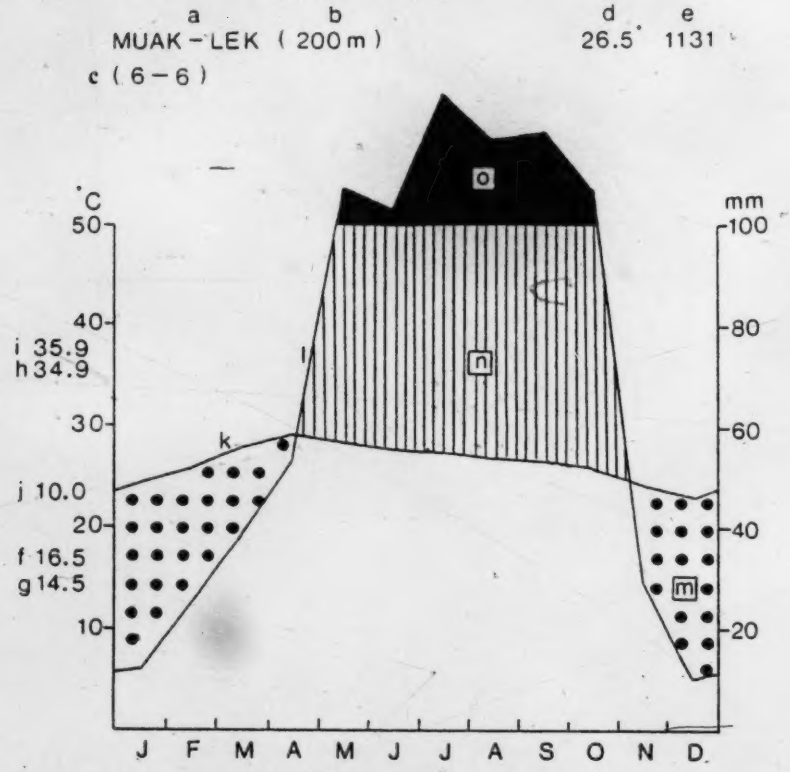

Figure 2. Climatatic diagram of location where seed collection area was obtained (data from Thai Danish Diary Farm, 1974-1979).
The letters and numbers on the diagram indicate the following: a, station; b, height above sea level; c, number of years of observation (where two figures are given, the first indicates temperature and the second precipitation); d, mean annual temperature; e, mean annual precipitation; f, mean minimum temperature of the coldest month; g, absolute minimum temperature (lowest recorded); h, mean maximum temperature of the warmest month; i, absolute maximum temperature (highest recorded); j, mean daily temperature fluctuation between day and night; k, curve of mean monthly temperature (1 division = 10°C); l, curve of mean monthly precipitation (1 division= 20 mm); m, period of relative drought (dotted); n, period of relative humid season (vertical shading); o, mean monthly precipitation over 100 mm (scale reduced to 1/10, black area).

2.2 Fruit and Seed Collection

Ten trees which were sampled grew along the bank of a creek. While the precise age of the trees has not been determined, they were estimated to be about 15 years old. Fruits and seeds usually develop in the period from June to October. These fruit and seed collection-times are shown in Table 1. Two hundred pods per tree were collected randomly from all positions of the crown with the pods bulked together during each collection time.

Table 1. Dates when fruits and seeds were collected in the school yard at Muak-Lek in 1985.

Number	Collection date	Days after anthesis
1	July, 15	30
2	July, 30	45
3	August, 15	61
4	August, 22	68
5	August, 29	75
6	September, 5	82
7	September, 12	89
8	September, 19	96
9	September, 26	103
10	October, 3	110

2.3 Seed Maturation and Germination

The following measurements were made for studying seed maturation at each collection time:

1. Pericarp, seed coat and funiculus color (Frequency-%).
2. Fruit moisture content (%).
3. Seed moisture content (%).

4. Seed fresh weight (mg).

5. Seed dry weight (mg).

Two hundred and forty pods were randomized from bulked pods. Half of these were measured to determine pericarp color and fruit moisture content. The other half was extracted with one seed per pod totalling 120 seeds. These seeds were then measured according to seed color, funiculus color, seed moisture content, seed fresh weight and seed dry weight. Color indexes of pericarp, seed coat and funicle were classified into three categories as shown in Table 2.

Table 2. Color indication of pericarp, seed coat and funicle based on previous observations.

Type	Color indication		
	1	2	3
Pericarp	Green	Greenish-brown	Brown
Seed coat	Green	Brown	Black
Funicle	Green	Yellow	Orange

Code 1 : early seed development
2 : middle seed development
3 : late development

Determination of fruit and seed moisture content follows the ISTA's rules (1976). The samples were weighed (fresh weight) in milligrams with a Sartorious analytical balance. The samples in containers were placed in an oven maintained at a temperature of 103±2°C and dried for 24 hours. At the end of the drying period, the samples were weighed again to determined dry weight. The moisture content

percentage was calculated based on its fresh weight by means of the following formula:

$$\% \text{ M.C.} = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Fresh wt.}} \times 100$$

Determination of seed fresh weight and dry weight was obtained during moisture content measurement.

The other measurement of maturation was the germination test. Germination tests were carried out from the 5th to the 10th collection time following the ISTA's rules (1976) as well. Four hundred seeds were counted at random from the bulked seeds which were extracted from the bulked pods. These seeds were divided into 4 replicates of 100 seeds per replication. Seed samples were X-ray radiographed to examine seed viability.[4] Later, four replicates of seeds were placed onto moist Kimpak paper in germination boxes. Seeds were germinated in a Conviron room germinator at the ASEAN-Canada Forest Tree Seed Centre. The temperature was maintained at a constant 30°C. Cool white fluorescent light was used to expose seeds for eight hours a day. Seed bed moisture was maintained by watering to meet the requirement for germination. Germination was counted daily for a period of 21 days. A seed was considered germinated when the emerging

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[4]X-ray radiography was taken with Kodak Industrex AA film within cabinet X-ray system, Fxitron series, Hewlett Packard model 43804N. The exposure condition was 30 seconds at 20Kv.

radicle of the seedling was four times the length of the seed. At the end of the germination test, ungerminated seeds were examined by dissection. The seeds that were firm were classified as ungerminated. Also seeds which were obviously dead or showed signs of abnormal germination were counted and removed during the period of the test and at the end of the test. These seeds were classified as dead seeds.

As previously mentioned, seeds were X-ray radiographed before germination. The internal characteristics of the seeds from the X-ray radiographs were classified into three categories with the following assumptions:

1. Mature seed : the embryo content was white and without space between seed coat and embryo.
2. Immature seed : the seed content showed either partly or fully white embryo with a space between seed coat and embryo.
3. Rotten seed : the seed showed traces of black spots.

The rest of the bulked seeds, from the 6th to the ~~10th~~ collection, were sent to Canada for further experiments on seed germination and dormancy. Two months after each collection date, 400 seeds from each collection which were stored at ambient temperatures, were sampled to test germination. Seeds were divided into 4 replicates of 100 seeds per replication. The germination conditions were the same as before. Moisture contents of seeds for each collection were determined with 10 replicates of 5 seeds per

replication.

2.4 Development of the Seed Coat

Basal flowers which had nearly the same size were marked in May 1985. Flowers, fruits and seeds were collected from June 15th (day of anthesis) to October. The paraffin method was used to illustrate the structures of the seed coat. Specimens were fixed in 50% Formalin-Acetic acid-Alcohol (FAA) (Johansen 1940), dehydrated with Tertiary Butyl Alcohol (TBA) series (Johansen 1940; Sass 1958), infiltrated and embedded in Paraplast[5] (m.p.= 56°C). The procedures for fixation, dehydration and infiltration are shown in the Appendix 1.

Thick sections (10-15 μm) were made with a rotary microtome, affixed to glass slides with Haupt's adhesive (Haupt 1930) and 4% formalin (Sass 1958; Gunn 1962), dried at 40-50°C for a short time, and left overnight. Sections were stained with Safranin O - Fast green (Johansen 1940), Delafield's Hematoxylin - Orange G or Periodic acid - Schiff's reaction (PAS) - 0.2% Light green SF (O'Brien and McCully 1981). Light photomicrographs of structures of the seed coat were made with the use of a Zeiss Photomicroscope (Department of Botany, the University of Alberta).

Seed coat characteristics play a major role in the germination of seeds of Leguminosae. As a result, morphometries on seed coat characteristics (the thickness of seed coat and the thickness of macrosclereid plus cuticular

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[5]Production by Monoject Scientific, Division of Sherwood Medical, St. Louis, U.S.A.

layer) were measured approximatly at the middle on the flat sides of seed with an ocular micrometer. The ocular micrometer was first calibrated with a stage micrometer for the Zeiss photomicroscope. The procedure for calculation the thickness is shown in the Appendix 2.

2.5 Seed Coat Treatment and Germination

The germination responses by seed coat treatments were considered in two ways as follows:

2.5.1 Effect of various physical and chemical treatments

One seed lot from a January 1985 collection at the Klang-Dong plantation was used for an experiment on seed coat scarification. The seeds were obtained from the ASEAN-Canada Forest Tree Seed Centre. The Klang-Dong plantation is about 10 km east of the centre. The reason why the Klang-Dong seed source was used instead of the Muak-Lek seed source, is that there were not enough seeds of the Muak-Lek source. These seeds were kept in the storage room at 4°C before testing germination in November 1985. Seeds were first X-ray radiographed to select sound seeds.[‘] Two thousand sound seeds were sampled from the bulk lot. Nine treatments plus control were tested. They were:

‘X-ray radiography was taken with Kodak X-Omat TL film within TFI cabinet X-ray system model M11NH at the University of Alberta. The exposure condition was 25 seconds at 15 Kv.

Control.
95°C water for 1, 3 and 5 minutes with shaking.
Concentrated sulfuric acid (H_2SO_4) for 5, 15 and 30 minutes with shaking.
Concentrated hydrochloric acid (HCl) for 15 minutes.
10% potassium hydroxide (KOH) for 24 hours.
Hand scarification (chipping the seed coat off at the cotyledon end).

Each treatment had four replicates with 50 seeds per replicate. The volumes of water, conc. H_2SO_4, conc. HCl and 10% KOH were 80, 20, 20 and 20 ml per replicate, respectively.

2.5.2 Effect of seed maturation on germination

From the study of the seed coat scarification in section 2.5.1, the best standard method was then used to treat seeds collected from September 5 through October 3 two months after collection. Two hundred seeds from each collection were used to investigate germination response. They were divided into 4 replicates of 50 seeds each.

Both germination tests in section 2.5.1 and 2.5.2 were run with a Conviron germinator at the University of Alberta using the same germination conditions as described in section 2.3.

2.6 Analyses of Data

The data of this study from section 2.3 to 2.5 were analyzed as follows:

2.6.1 Seed maturation and germination

Frequency histogram of pericarp, seed coat and funicle color were determined for 120 seed samples in each collect The relationship between other seed maturity indices such as fruit moisture content, seed moisture content, seed fresh weight and seed dry weight and time of maturation were analyzed using regression procedures (Kleinbaum and Kupper 1978). The independent variable (maturation time) starts on day zero, approximately on June 15th, or at the time of anthesis. Transformations were used to stabilize the variance and to transform variables to the normal distribution of the dependent variable if the straight-line or polynomial regression model was not satified.

With analysis of germination, the percentages of germinated, ungerminated and dead seed were calculated and based on the number of mature plus immature seeds examined through X-ray radiographs in each replication. For example, if 100 seeds per replication were tested, and 50 seeds germinated, and if X-ray radiographs showed only 90% live seeds (mature plus immature seeds), then germination percentage of this replication would be 55.56%, not 50%.

The other germination response was germination rate. The germination rate was calculated as the number of days to achieve 50% of total germination over the duration of the

test.

One way analysis of variance was used to find an effect of seed collection time on seed viability, germination, and germination rates. If there were significant differences among the collection times, Student-Newmann-Kuels' multiple range test (S-N-K) at $P \leq 0.05$ was used to compare their means (Steel and Torrie 1980).

2.6.2 Development of the seed coat

The relationship between morphometric parameters and time of maturation was also analyzed with the use of regression analyses.

2.6.3 Seed coat treatments and germination

One way analysis of variance was performed to study the effect of seed coat treatments on germination, germination rate and seed collection time. If they were different, S-N-K multiple comparison procedures were used to compare means.

3. RESULTS

3.1 Seed Maturation and Germination

The results of maturity indices and germination response are given in separate sections as follows:

3.1.1 Color indices

Changes in the color of pericarp, seed coat and funiculus during maturation are presented in Figures 3-5. Pericarp color changed from entirely green through greenish-brown to entirely brown at the end of the maturing process. The change in color was distinctive; prior to July 30 all pericarps were green, then, on August 15, 22, 29, September 5 and September 12, respectively, 2, 20, 77, 88 and 100% of pericarps appeared brown. Likewise, seed coat color changed from green through brown to completely black over this same time period. Funiculus color changed faster than pericarp and seed coat color. Only 10% of the funicles were yellow on July 30, and over 80% were orange by August 15, and they remained unchange until September 5 when all funicles had turned orange.

The browning of the pericarps was associated with dehiscing along the longitudinal sutures of pods after August 15. Mostly black seeds were obtained from the greenish-brown and brown pods. Pods were completely dehiscent by September 12. Prior to this day, some seeds fell out of the brown pericarps and dropped to the ground

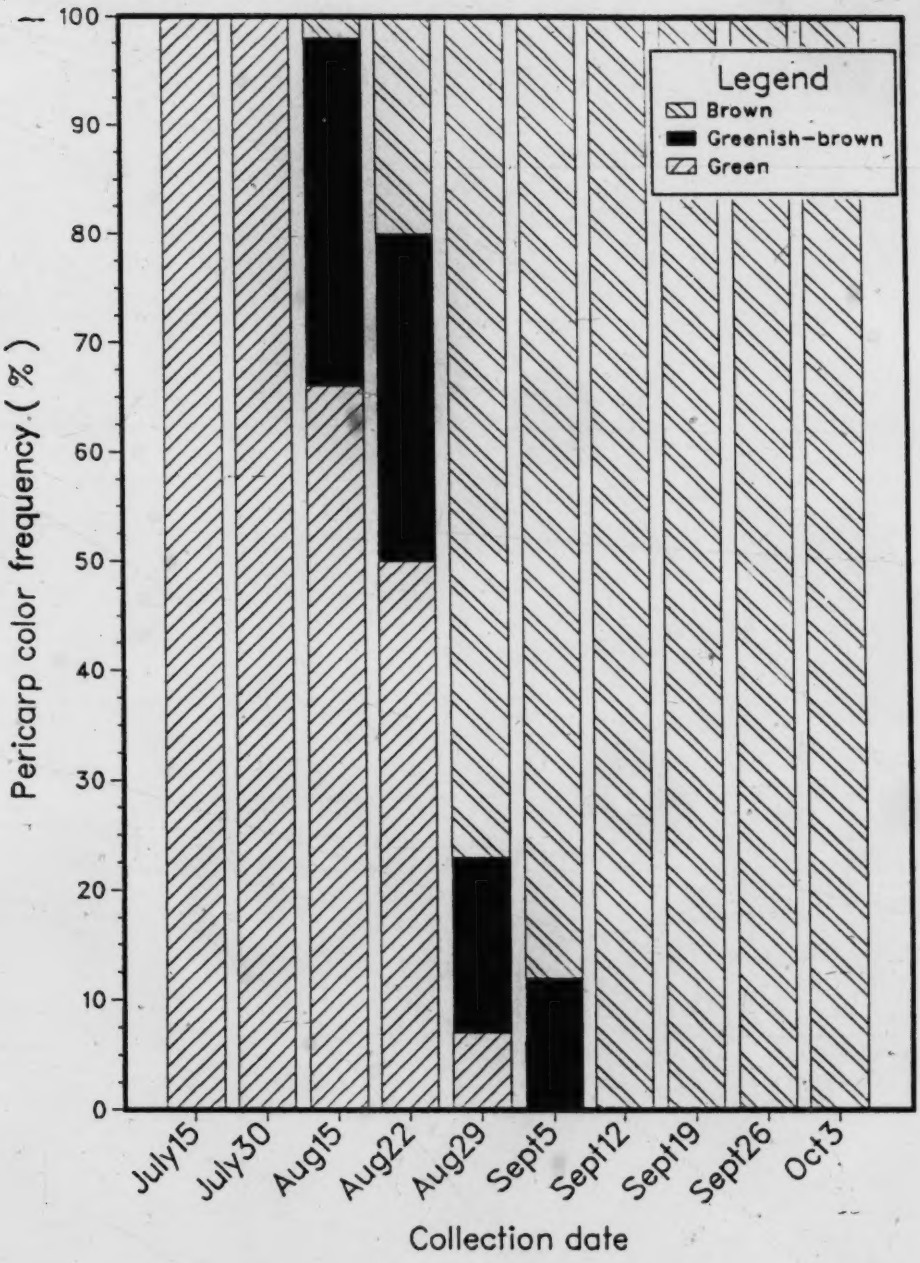

Figure 3. Changes in pericarp color during maturation.

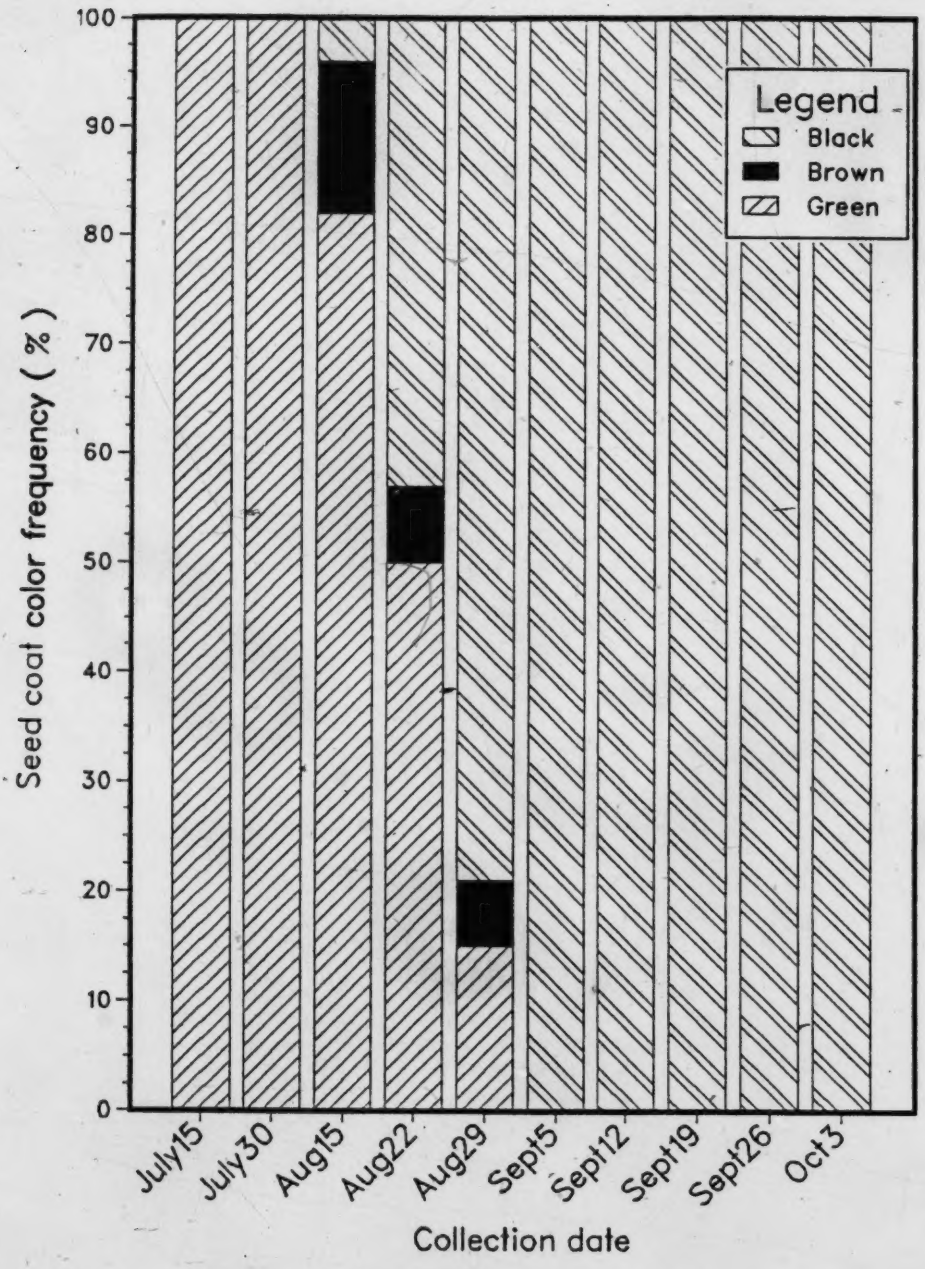

Figure 4. Changes in seed coat color during maturation.

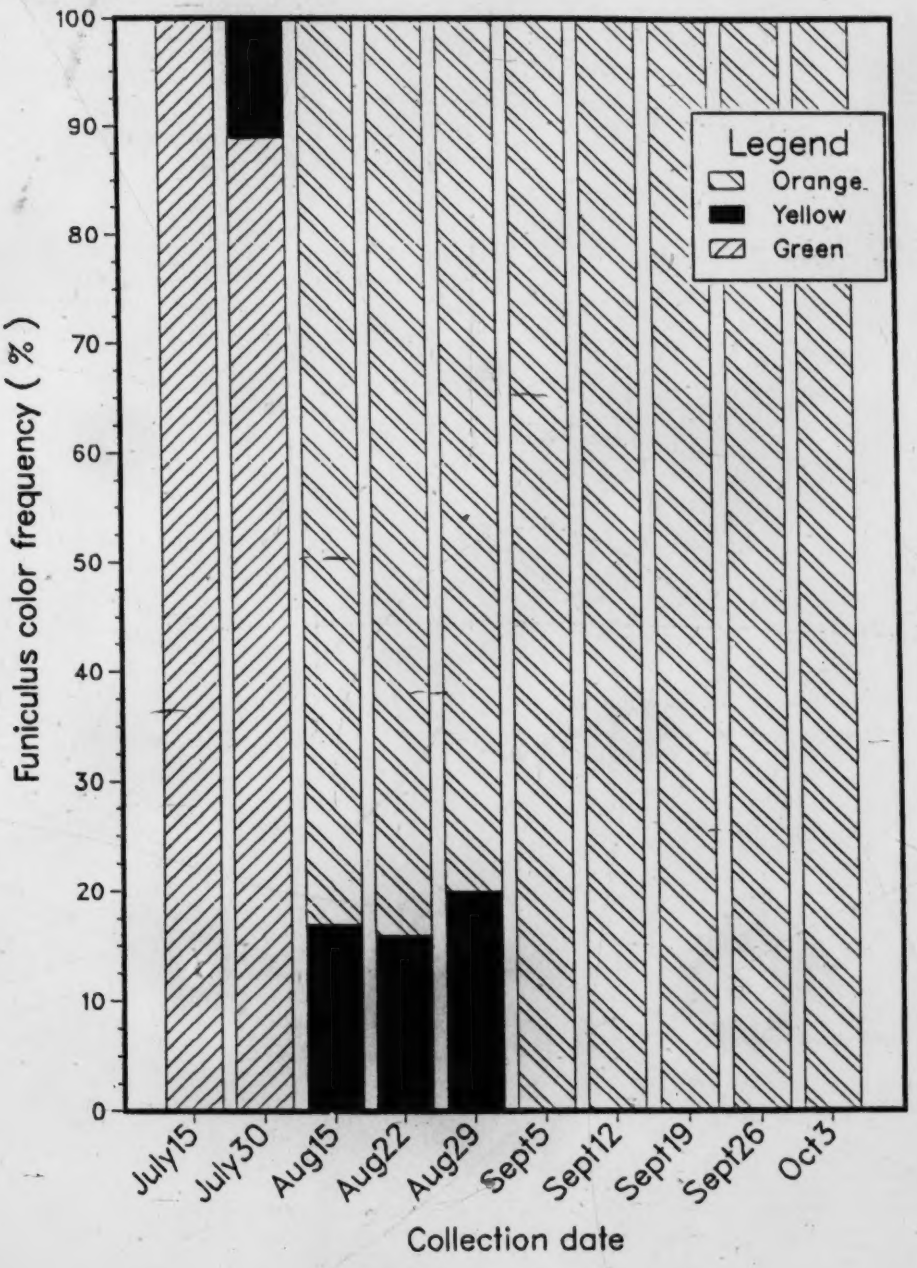

Figure 5. Changes in funiculus color during maturation.

but some were still attached to their pods by their funicles. Many more seeds dropped to the ground after September 19 and all seeds had been shed by October 6.

3.1.2 Fruit and seed moisture content

There was a significant decrease in fruit and seed moisture content during maturation. The regression equations and lines of fruit and seed moisture content are presented in Figure 6. Seed moisture content showed a higher correlation (R^2 = 0.945) than fruit moisture content (r^2 = 0.820). At the beginning, seed moisture content was higher by about 11% than fruit moisture content. Subsequently, seed moisture content dropped faster than fruit moisture content. However, there is no pronounced difference between fruit and seed moisture content. On the last two collections (September 26 and October 3) fruit and seed moisture contents stabilized at about 19 and 14%, respectively.

3.1.3 Seed fresh weight and dry weight

The relationship between seed fresh weight, seed dry weight and maturation is illustrated in Figure 7. A log transformation was used to increase the correlation between two variables and to stabilize the variance of dependent variables. Both seed fresh weight and dry weight showed high correlation with maturation (R^2 = 0.963 and 0.999, respectively). Both fresh weight and dry weight increased

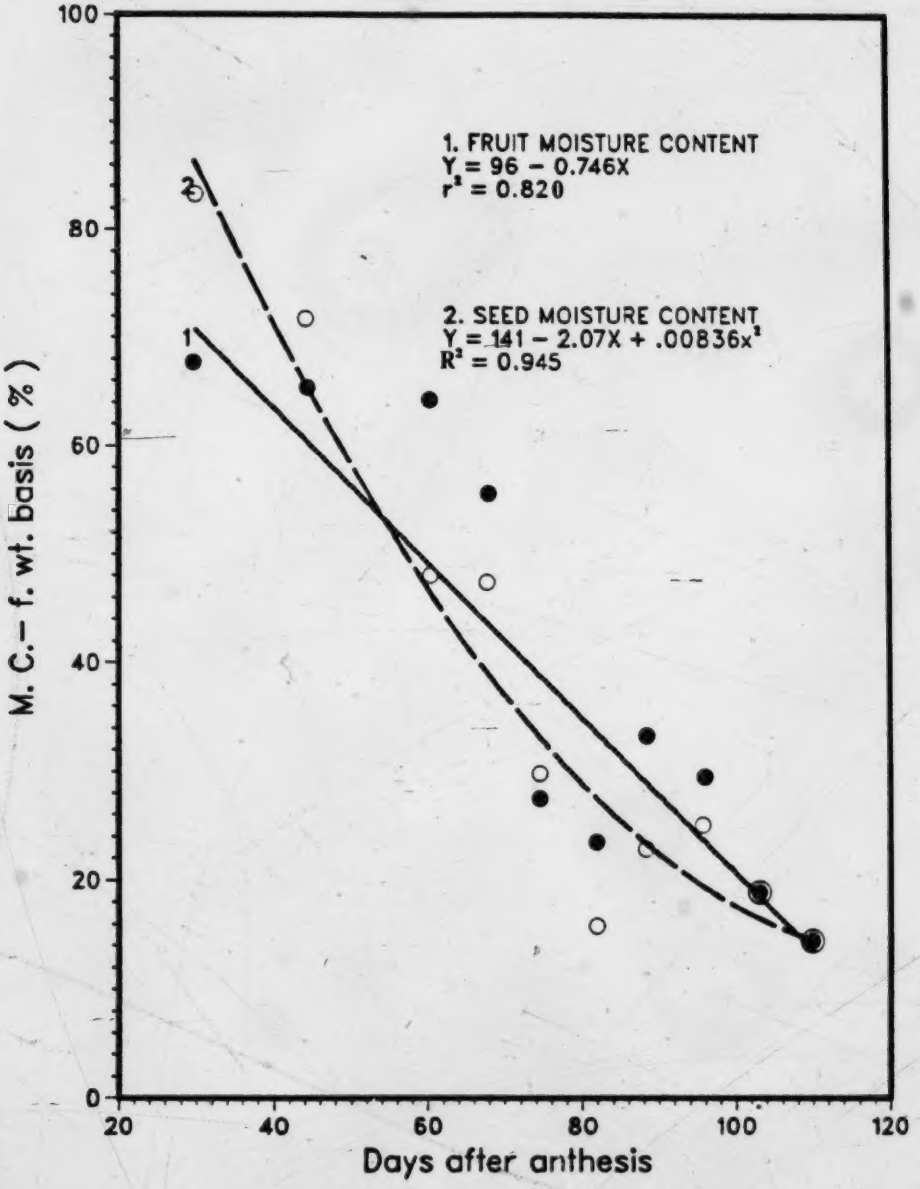

Figure 6. Changes in fruit and seed moisture content during maturation. ●————● : fruit moisture content. o------o : seed moisture content.

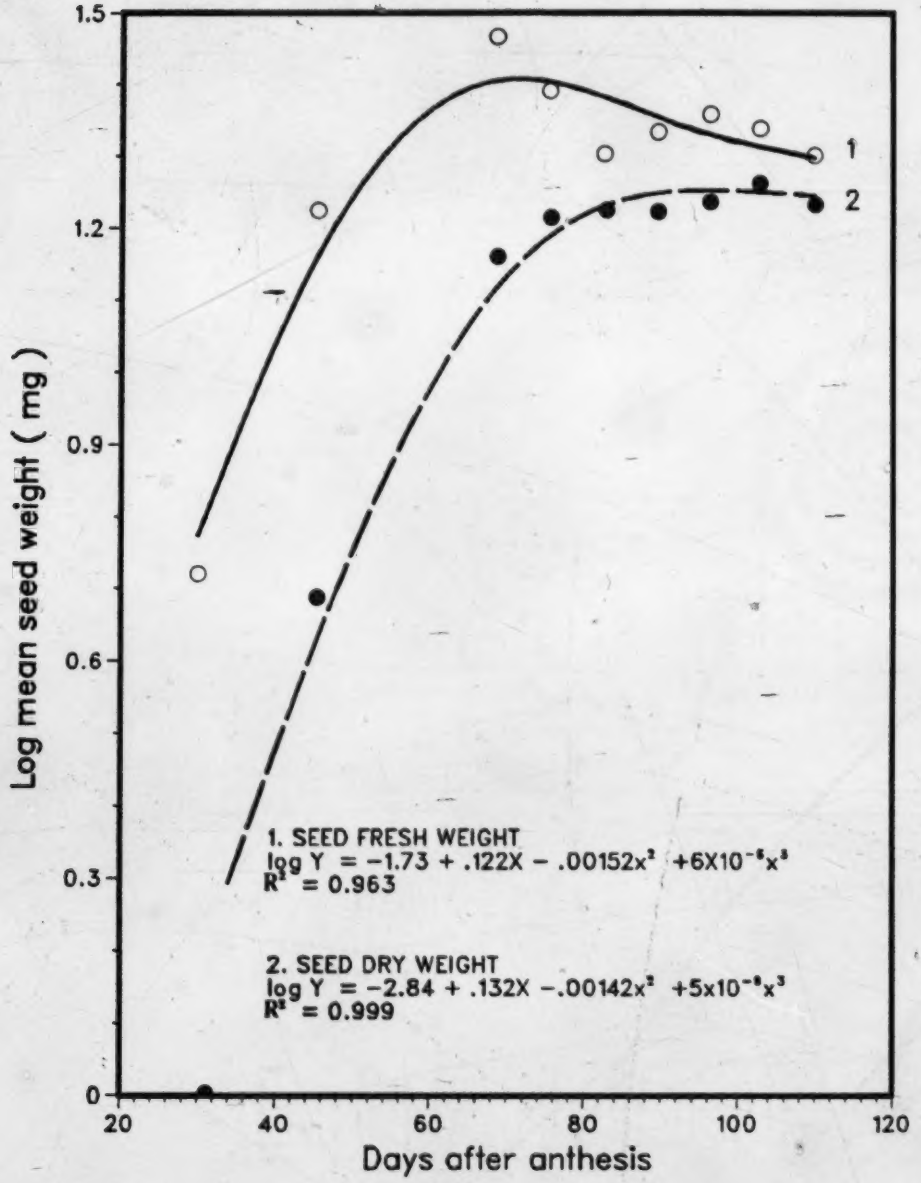

Figure 7. Changes in seed fresh weight and dry weight during maturation. ○———○ : seed fresh weight. ●------● : seed dry weight.

remarkably and were nearly parallel during the first 68 days of maturation. Seed fresh weight was 29.7±11.76 mg at the peak of day 68. Subesquently, seed fresh weight fell gradually due to continual loss of water content while seed dry weight still increased until maximal dry weight was reached. Thereafter, there was no change in dry weight from day 82 after anthesis (September 5) to October 3.

3.1.4 Seed viability

Viability of seed analyzed from X-ray radiographs showed that most seeds were mature by August 29. However, analysis of variance showed significant differences among means of percent mature seed (Table 3). Since both mature and immature seeds can germinate analysis of variance for mature plus immature seeds was performed and is presented in Table 4. It also showed that seed viability still was significantly different among collection dates. The comparisons of mean seed viability for mature and mature plus immature seeds indicated that seeds collected on August 29 still had less viability than did seeds collected from September 5 to October 3. It also showed high variation in percent mature and immature seeds among samples collected on August 29 as shown by high standard deviations (Table 5). On average, mean percent seed viability for all collection dates for mature seeds ranged from 85.46% to 91.29% and for mature plus immature seeds ranged from 92.62% to 96.54% at 95% confident interval.

Table 3. Viability based on mature seeds from X-ray pictures analyzed by ANOVA.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	878.88	175.78	14.73	.0000
Within groups	18	214.75	11.93		
Total	23	1093.63			

Table 4. Viability based on mature plus immature seeds from X-ray pictures analyzed by ANOVA.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	362.83	72.57	9.82	.0001
Within groups	18	133.00	7.39		
Total	23	495.83			

Table 5. Comparisons for means of seed viability among collection dates.

Collection date	Mature mean		Mature S.D.	Mature+Immature mean		Mature+Immature S.D.
Aug. 29	75.00	b	5.72	86.75	b	4.35
Sept. 5	90.00	a	1.29	95.00	a	2.16
Sept. 12	89.50	a	3.42	94.25	a	1.89
Sept. 19	91.00	a	3.74	94.75	a	3.77
Sept. 26	92.25	a	2.21	98.00	a	1.41
Oct. 3	92.00	a	2.58	98.75	a	0.96

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

3.1.5 Seed germination

Germination generally started on day 3 after sowing for seeds collected after September 19 and on day 4 for seeds collected on August 29 to September 12. Total germination increased gradually and was almost completed by day 21 for all collection dates. The representative proportion of germinated, ungerminated and dead seed is shown in Figure 8. Percent total germination increased from August 29 (day 75) and reached its maximum on September 12 (day 89). Thereafter, it decreased with later collection. Analysis of variance for percent total germination showed significant difference among collection dates (Table 6). Multiple comparisons for mean total germination indicated that germination by September 12 was superior to all other collections (Table 8). Germination on August 29 was much more variable among replication than the remainders as shown by its high standard deviation (Table 8).

The regression models to demarcate the area of germinated, ungerminated and dead seed (Figure 8) are shown as follows:

1. The regression equation for the area of germinated seed is:

$$Y = -304 + 8.44X - .0475X^2$$

$R^2 = 0.705$

Where Y = percent germination
X = days after anthesis
$n = 24$

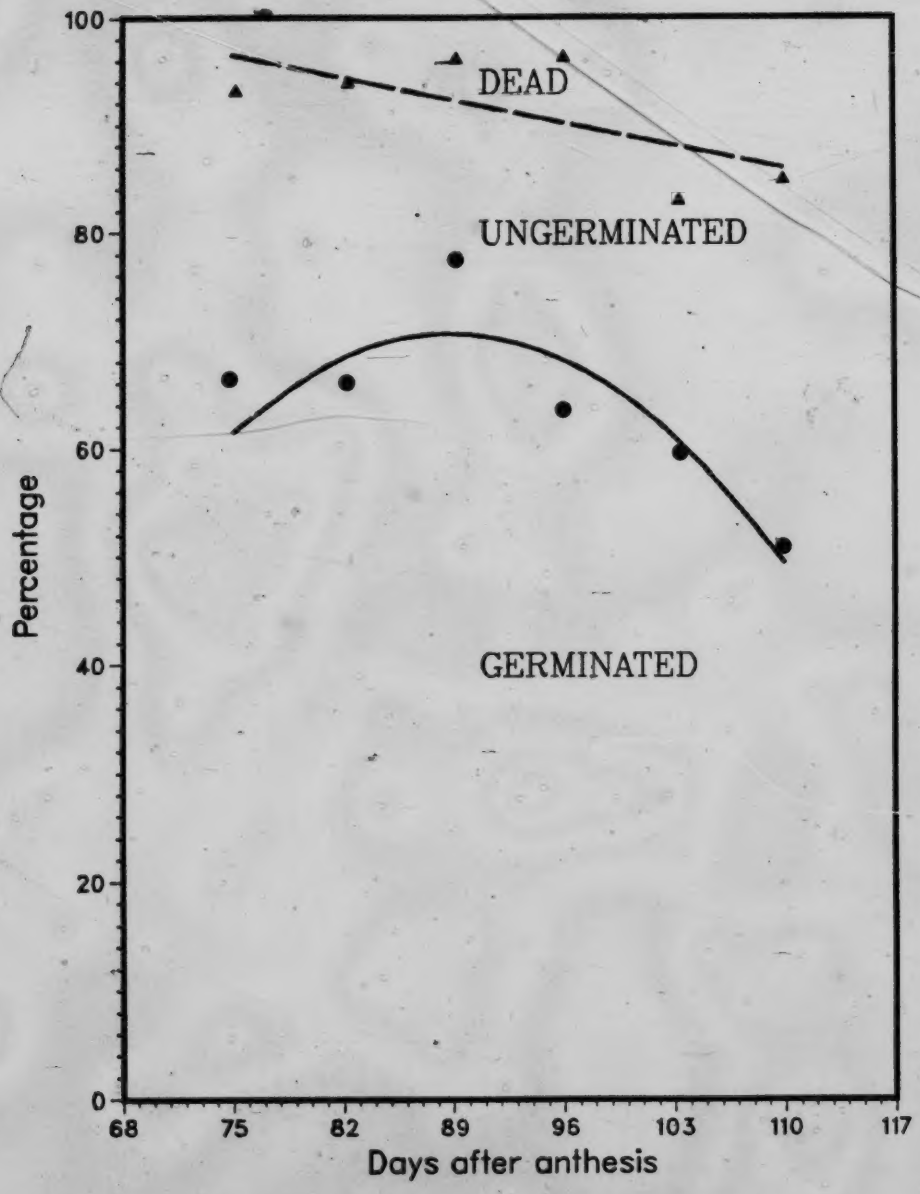

Figure 8. The proportion of seed which germinated, would not germinated and dead seed in relation to the time of collection.
●———●: Mean germinated seed.
▲– – –▲: Mean germinated plus ungerminated seed.

2. The regression equation defining the boundary between the area of ungerminated and dead seed is:

$$Y = 119 - 0.3X$$
$$r^2 = 0.308$$

Where
Y = percent germ. + ungerm. seed
X = days after anthesis
n = 24

Analysis of variance for ungerminated and dead seeds showed significant differences among the various collection dates (Tables 7 and 8). Multiple comparisons of their means are presented in Table 9. The overall percentage of germinated, ungerminated and dead seed for this 1985 collections are tabulated in Table 9.

Table 6. ANOVA for seed germination at the time of collection among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	1528.38	305.68	10.00	.0001
Within groups	18	550.22	30.57		
Total	23	2078.59			

Table 7. ANOVA for ungerminated seed at the time of collection among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	602.78	120.56	5.19	.004
Within groups	18	418.12	23.23		
Total	23	1020.90			

Table 8. ANOVA for dead seed at the time of collection among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	703.85	140.77	8.43	.0003
Within groups	18	300.65	16.70		
Total	23	1004.50			

Table 9. Comparisons of mean percent germinated, ungerminated and dead seed among collection dates.

Collection date	Germinated Mean		S.D.	Ungerminated Mean		S.D.	Dead Mean		S.D.
Aug. 29	66.37	b	10.94	26.33	ab	6.81	7.30	a	4.21
Sept. 5	66.29	b	4.21	27.69	ab	7.61	6.02	a	3.65
Sept. 12	77.42	a	2.55	19.11	a	1.02	3.47	a	1.83
Sept. 19	63.66	b	3.58	32.95	b	2.56	3.39	a	3.66
Sept. 26	59.68	b	4.06	23.71	ab	1.42	16.61	b	4.94
Oct. 3	50.87	c	3.22	33.45	b	5.04	15.68	b	5.29
Total	64.05		9.51	27.20		6.66	8.75		6.61

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

The rate of germination exhibited a polynomial regression pattern related to seed maturation (Figure 9). Analysis of variance for germination rates revealed significant differences among seed collection dates (Table 10). Multiple comparisons for mean germination rates indicated that seeds collected on September 19 and 26 showed the best performance of 4.98 and 4.62 days, respectively (Table 11). Seeds collected on August 29 germinated slowly (R50= 9.56 days).

Table 10. ANOVA for germination rate at the time of collection among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	5	61.87	12.37	25.46	.0000
Within groups	18	8.75	0.49		
Total	23	70.62			

Table 11. Comparisons for means of germination rate among collection dates.

Collection date	Mean (days)	S.D. (days)	Comparison of means
Aug. 29	9.56	1.14	c
Sept. 5	6.45	0.35	b
Sept. 12	6.93	1.07	b
Sept. 19	4.98	0.22	a
Sept. 26	4.62	0.19	a
Oct. 3	6.24	0.51	b

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

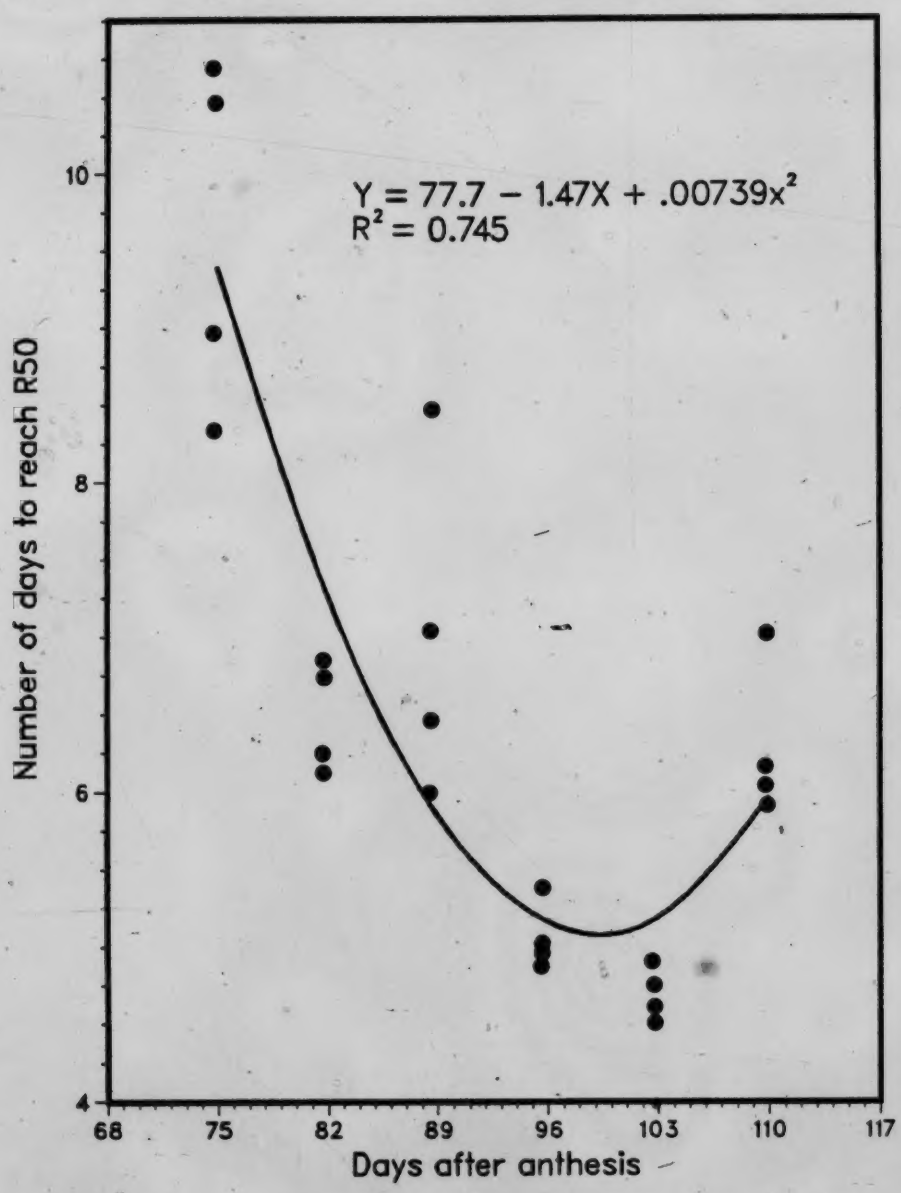

Figure 9. Germination rate at the time of collection.

3.1.6 Germination two months after collection

Two months after seed collection, all seed collections began to germinate from day 4 to day 6. The percent germination increased slightly over the 21-day test duration. The representative proportion of percent germinated, ungerminated and dead seeds from September 5 to October 3 was illustrated in Figure 10. The regression models for this figure are shown as follows:

1. The regression equation defining the boundary between the area of germinated and ungerminated seed is:

$$Y = -22.45 + .475X$$

$r^2 = 0.325$

Where Y = percent germination.
X = days after anthesis.
$n = 20$

2. The regression equation to demarcate the boundary between ungerminated and dead seed is:

$$Y = 115 - .286X$$

$r^2 = 0.306$

Where Y = percent germ. + ungerm.
X = days after anthesis.
$n = 20$

The regression model for germination showed a low correlation with maturation. However, the trend of germination seems to be low on early seed collection. Analysis of variance for mean germination showed significant

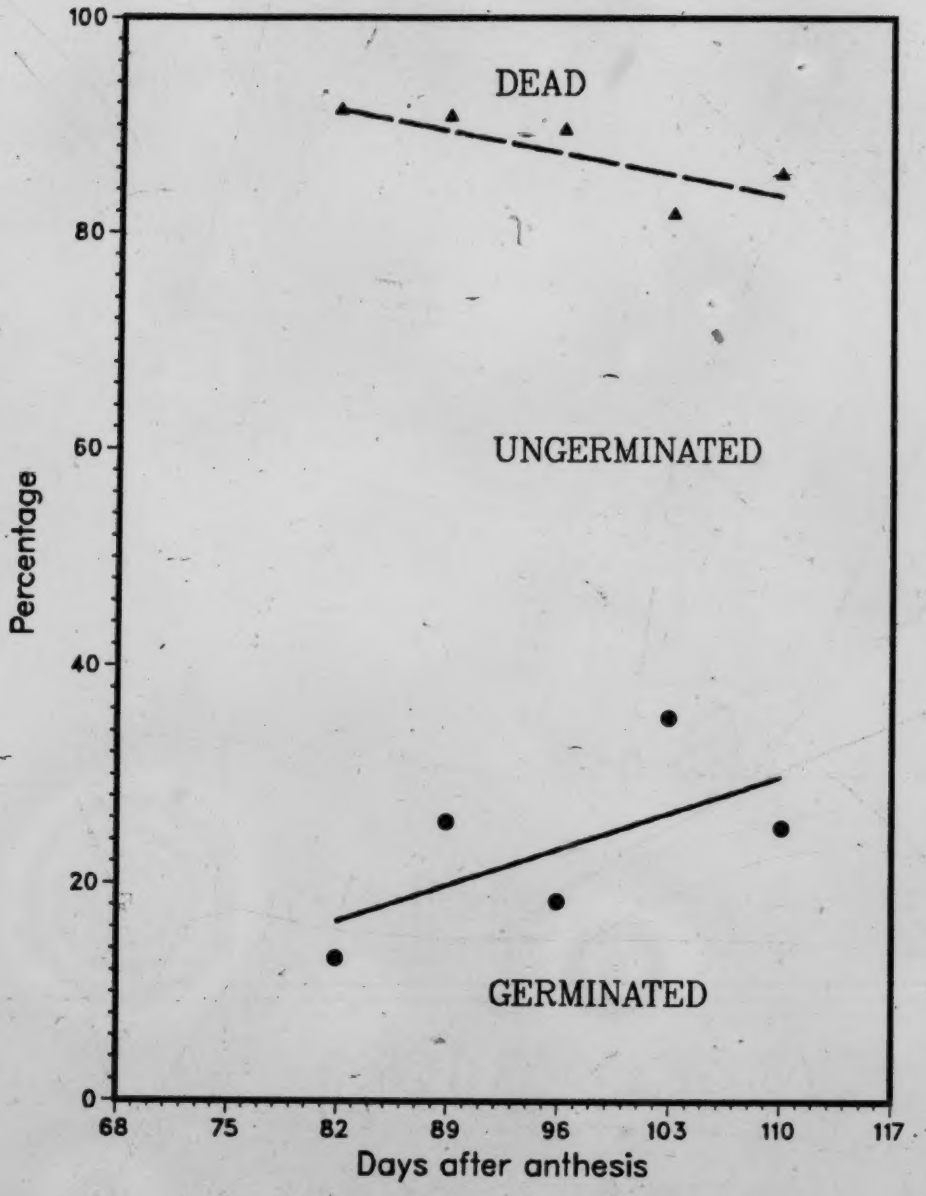

Figure 10. The proportion of seeds which germinated, would not germinated and dead seed two months after collection.
●——●: Mean germinated seed.
▲– – –▲: Mean germinated plus ungerminated seed.

differences among collection dates (Table 12). Multiple comparisons for mean germination showed that seeds collected on September 26 had high germination and differed from the others (Table 15).

Table 12. ANOVA for germination two months after collection among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	1182.30	295.58	24.59	.0000
Within groups	15	180.25	12.02		
Total	19	1362.55			

The regression model for two month old seeds for germinated plus ungerminated seed also showed a low correlation with maturation. Contrary to the germination regression, it showed a negative slope. Seeds collected early tended to have higher percent germinated plus ungerminated seed than those of later collections. Analysis of variance for ungerminated seed also showed highly significant differences among collection dates (Table 13). Multiple comparisons for mean ungerminated seed indicated that seeds collected on September 5 did not differ significantly from seeds collected on September 19, but differed significantly from those of the remainders, particularly on September 26 which had the least ungerminated seeds (Table 15). Analysis of variance for dead seed also showed significant differences at $P \leq 0.05$ among

collection dates (Table 14). Multiple comparisons for mean dead seeds are presented in Table 15. When considering the total data between two germination tests (at the time of collection and two months later), mean germination dropped remarkably from 64% to 23% while mean ungerminated seed increased greatly from 27% to 64% and mean dead seed increased slightly from 8.75% to 12.55% over the period two months after collection (Tables 9 and 15).

Table 13. ANOVA for ungerminated seed two months after collection.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	2356.7	589.18	20.97	.0000
Within groups	15	421.5	28.10		
Total	19	2778.2			

Table 14. ANOVA for dead seed two months after collection.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	254.70	63.68	3.56	.0311
Within groups	15	268.25	17.88		
Total	19	522.95			

Table 15. Comparisons for mean germinated, ungerminated and dead seed two months after collection among collection dates.

Collection date	Germinated MEAN	S.D.	Ungerminated MEAN	S.D.	Dead Mean	S.D.
Sept. 5	13.00 c	2.16	77.75 a	5.32	9.25 b	6.40
Sept. 12	25.00 b	1.41	65.50 bc	1.29	9.50 b	1.91
Sept. 19	17.75 c	2.87	71.50 ab	1.29	10.75 ab	2.87
Sept. 26	35.75 a	3.30	45.75 d	7.80	18.50 a	5.00
Oct. 3	24.25 b	5.85	61.00 c	6.93	14.75 ab	3.40
Total	23.15	8.47	64.30	12.09	12.55	5.25

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

Germination rate also changed remarkably from the polynomial to the straight-line regression with the negative slope and high correlation ($r^2 = 0.95$) (Figure 11). All seed collections showed a delayed germination rate with a sharp decrease (9.80 days) on September 5 and gradually less decreases for the later collections (Tables 11 and 17). Analysis of variance showed that germination rate differed significantly among collection dates (Table 16). Multiple comparisons for mean germination rate indicated that seeds collected on October 3 had the best germination rate and differed significantly from all other collections (Table 17).

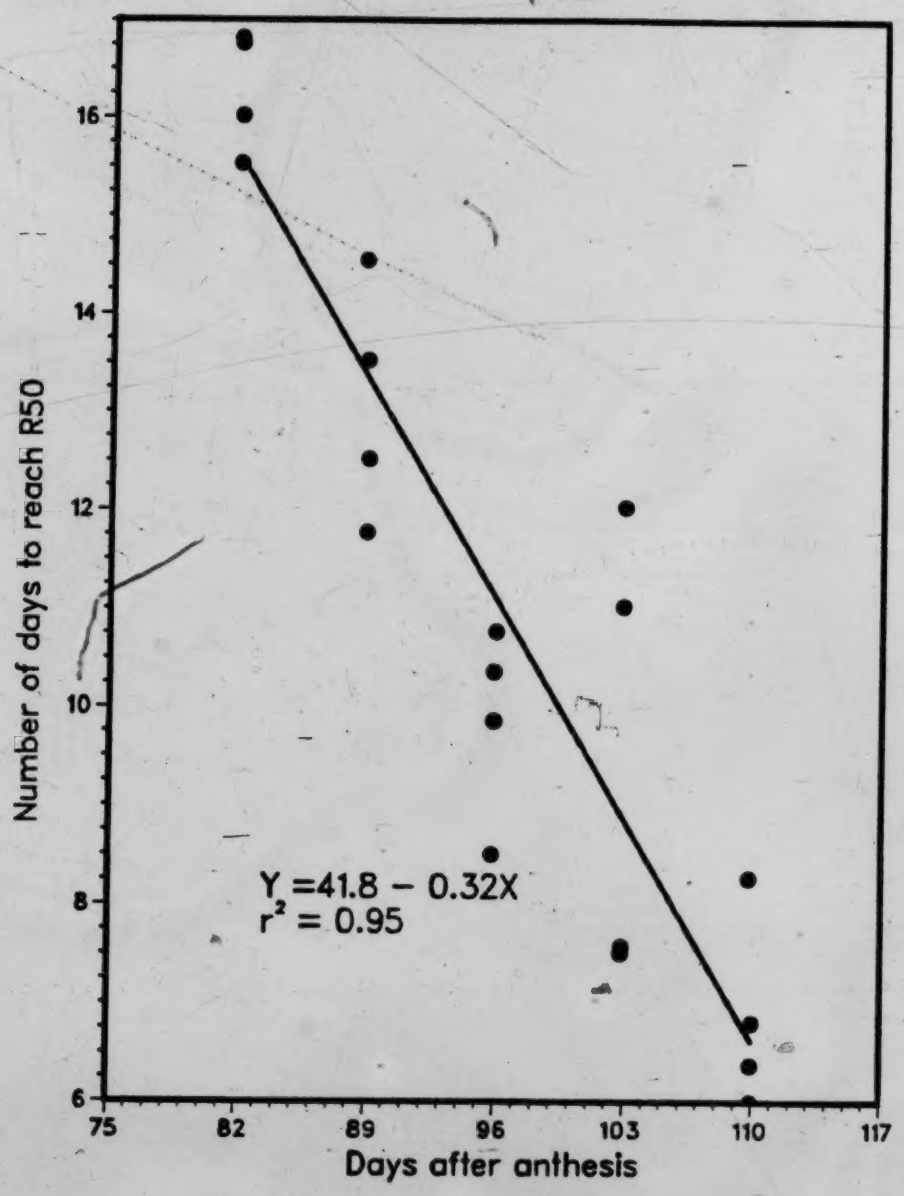

Figure 11. Germination rate two months after collection.

Table 16. ANOVA for germination rate two months after collection.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	211.54	52.89	28.28	.0000
Within groups	15	28.00	1.87		
Total	19	239.54			

Table 17. Comparisons for mean germination rate two months after collection.

Collection date	Mean (days)	S.D.	Comparison of means
Sept. 5	16.25	0.61	d
Sept. 12	13.06	1.20	c
Sept. 19	9.81	0.98	b
Sept. 26	9.48	2.36	b
Oct. 3	6.83	1.00	a

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

Two months after collection, seed moisture content dropped to around 8 to 10% for all collections (Table 19). However, analysis of variance for moisture content showed significant differences among collection dates (Table 18). Multiple comparisons for mean moisture content showed that the moisture content did not differ significantly among seeds collected from September 5 to September 26(Table 19).

Table 18. ANOVA for percent seed moisture content two months after collection.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	17.03	4.26	4.97	.0021
Within groups	45	38.53	0.86		
Total	49	55.56			

Table 19. Comparisons for mean percent seed moisture content two months after collection.

Collection date	Mean (%)	S.D.	Comparison of means
Sept. 5	9.55	0.65	ab
Sept. 12	8.68	0.81	a
Sept. 19	8.91	1.12	a
Sept. 26	8.61	1.26	a
Oct. 3	10.13	0.62	b

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

3.2 Seed Coat Structure and Development

3.2.1 Ovary and ovule

The ovary is superior and is covered by uniseriate hairs. The ovary began expansion during the first week after anthesis. The nucellus of the ovules are not covered completely with integuments at the beginning of fruit development (Figure 12). The mature ovule is crassinucellar, bitegmic and anatropous with a long funicle (Figure 13). Both the outer and inner integuments are bilayered at the beginning (Figure 12). Subsequently, the outer integument grows in many cells thick while the inner integument remains two cells thick; however, the inner integument may be three cells thick (Figure 14). The micropyle is straight and formed by the outer integument alone. The pattern of ovular venation of this species is the simplest type as found in Mimosoideae; the raphe-bundle extends as a curved and unbranched vein through the chalaza and the outer integument and nearly to the micropylar end (Figure 14). With the enlargement of the embryo sac, the nucellus and the inner integument become crushed (Figure 14).

3.2.2 Seed coat

The seed coat is formed by the outer integument. The cells of the superficial layer of the outer integument show the typically anticlinal divisions while the cells of the inner layers have both anticlinal and periclinal divisions, inferred by observing cell shapes. The immature seed coat

has two zones: (i) the epidermal single layer of macrosclereids and (ii) multi-layers of parenchyma (Figures 15 and 16). The seed coat structures show this pattern until the end of July. Subsequently, when the seed is more mature, the seed coat is differentiated into five zones: from outside to inside (i) cuticular layer, (ii) macrosclereid layer, (iii) hypodermal osteosclereid layer, (iv) parenchyma layers, and (v) inner osteosclereid layer (Figures 17-21).

The macrosclereid cells are cuboidal cells, except at the micropylar end, during early development (Figure 15). The macrosclereid cells at the micropylar end develop faster than those of the remainders as shown by longer cells (Figure 15). However, the length of macrosclereids in the hilar region does not differ strongly from those in the remaining regions when they become mature (Figure 20). Thereafter, macrosclereid cells elongate radially with much more cuticular deposition covering the caps of the macrosclereids on the seed coat surface. The thickness of macrosclereid plus cuticular layer showed a close relationship to seed maturation with a high correlation (R^2= 0.912) (Figure 24). The macrosclereid plus cuticular layer increased rapidly in thickness during the two months following anthesis. Afterwards, the layer increased slightly to a maximal thickness of 79 μm on day 82 after anthesis. Subsequently, it started to decrease in thickness to about 69-79 μm when the seeds were mature.

The hypodermal and inner osteosclereid cells are differentiated, respectively, from the hypodermal and

innermost parenchyma cells, and appear well-defined in early August (Figure 17). However, the hypodermal osteosclereid cells are not differentiated at the hilar region (Figure 20). In addition, the innermost layer sometimes develops some macrosclereid cells instead of osteosclereid cells (Figure 21). Both the hypodermal and inner osteosclereid layers persist when the seeds are mature (Figure 23).

Generally, the parenchyma cells of the immature seed coat are nearly isodiametric (Figures 15 and 16). Subsequently, they enlarge, elongate tangentially, and have wall thickening particularly on the tangential walls (Figures 19 and 22). Thereafter, the parenchyma cells in the inner layers (only 1-3 layers) are crushed (Figure 22). The remaining parenchyma cells have progressively smaller cell cavities as they mature. At last, the parenchyma cells are completely compact without cell cavities (Figure 23). The seeds collected between September 5 and October 3 show the typically compressed parenchyma cells. The parenchyma cells below the macrosclereid cells at the hilum do not elongate. They are still small, dense and thick-walled (Figure 20).

The seed coat thickness of the immature seed is uneven as shown by the seed coat on its flat sides; here they are thinner than where the vascular bundles are or along the seed edges (Figure 16). When the seed is more mature, the thickness of seed coat is more uniform over an entire seed (Figure 18), except at the hilar region. The thickness of the seed coat increased rapidly during early seed maturation. The seed coat attained a maximum thickness

(261-264 μm) on day 55-61 after anthesis (Figure 25). Afterthis, the thickness of seed coat decreased due to the collapsing of the parenchyma cells until nearly constant (184-213 μm) on the last five collections.

Abbreviations used for Figures 12-23:

ch	:	chalaza
co	:	cotyledon
cu	:	cuticle
eap	:	egg apparatus
em	:	embryo
ems	:	embryo sac
en	:	endosperm
fu	:	funicle
ho	:	hypodermal osteosclereid
ii	:	inner integument
io	:	inner osteosclereid
ll	:	light line
mc	:	macrosclereid cell
mi	:	micropyle
nu	:	nucellus
oi	:	outer integument
ov	:	ovule
pc	:	parenchyma cell
pe	:	pericarp
pl	:	plumule
ren	:	remnant endosperm
sc	:	seed coat
vb	:	vascular bundle

Figure 12. The ovule in the cavity of the fruit showing early development of integuments. The fruit was collected on June 18, 1985. The section was stained with Safranin O - Fast green. X80.

Figure 13. The mature ovule in the fruit showing an anatropous ovule with long funicle. The fruit was collected on June 23, 1985. Section was stained with Safranin O - Fast green. X19.69.

Figure 14. Enlargement of the mature ovule from Figure 13 showing details of the ovule. The section was stained with Safranin O - Fast green. The arrow shows crushed nucellus. X100.

Figure 15. The immature seed showing zonations of seed coat and macrosclereid cells at the micropylar end. The specimen was collected on June 28, 1985. The section was stained with Delafield's Hematoxylin - Orange G. X31.25.

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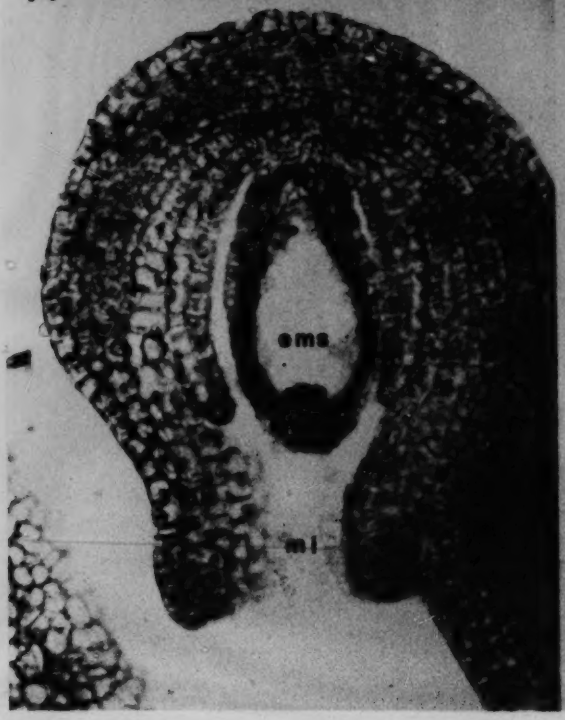

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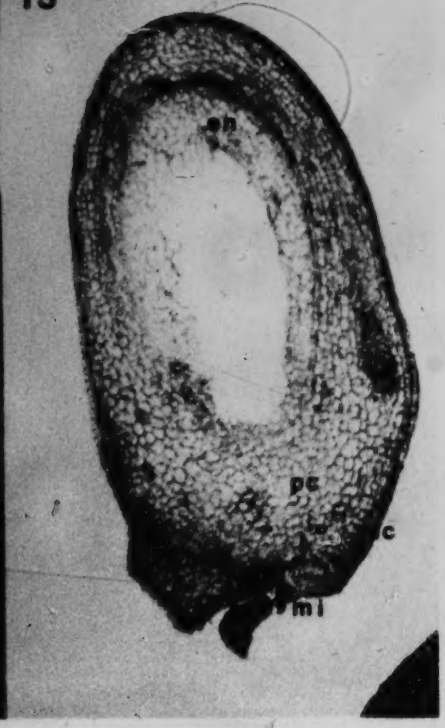

Figure 16. Cross section of immature seed showing zonations and thickness of the seed coat. The specimen was collected on July 17, 1985. The section was stained with Safranin O - Fast green. The arrow indicates plasmolysis of endosperm due to the FAA fixative. X19.69.

Figure 17. Cross section of the developing seed coat showing differentiation of the seed coat into 5 zones. The specimen was collected on August 2, 1985. The section was stained with Safranin O - Fast green. X40.

Figure 18. Cross section of the early mature seed showing the even thickness of the seed coat. The specimen was collected on August 15, 1985. The section was stained with Delafield's Hematoxylin - Orange G. X7.8.

Figure 19. Enlargement of the seed coat from Figure 18 showing well-defined zonations of the seed coat. X40.

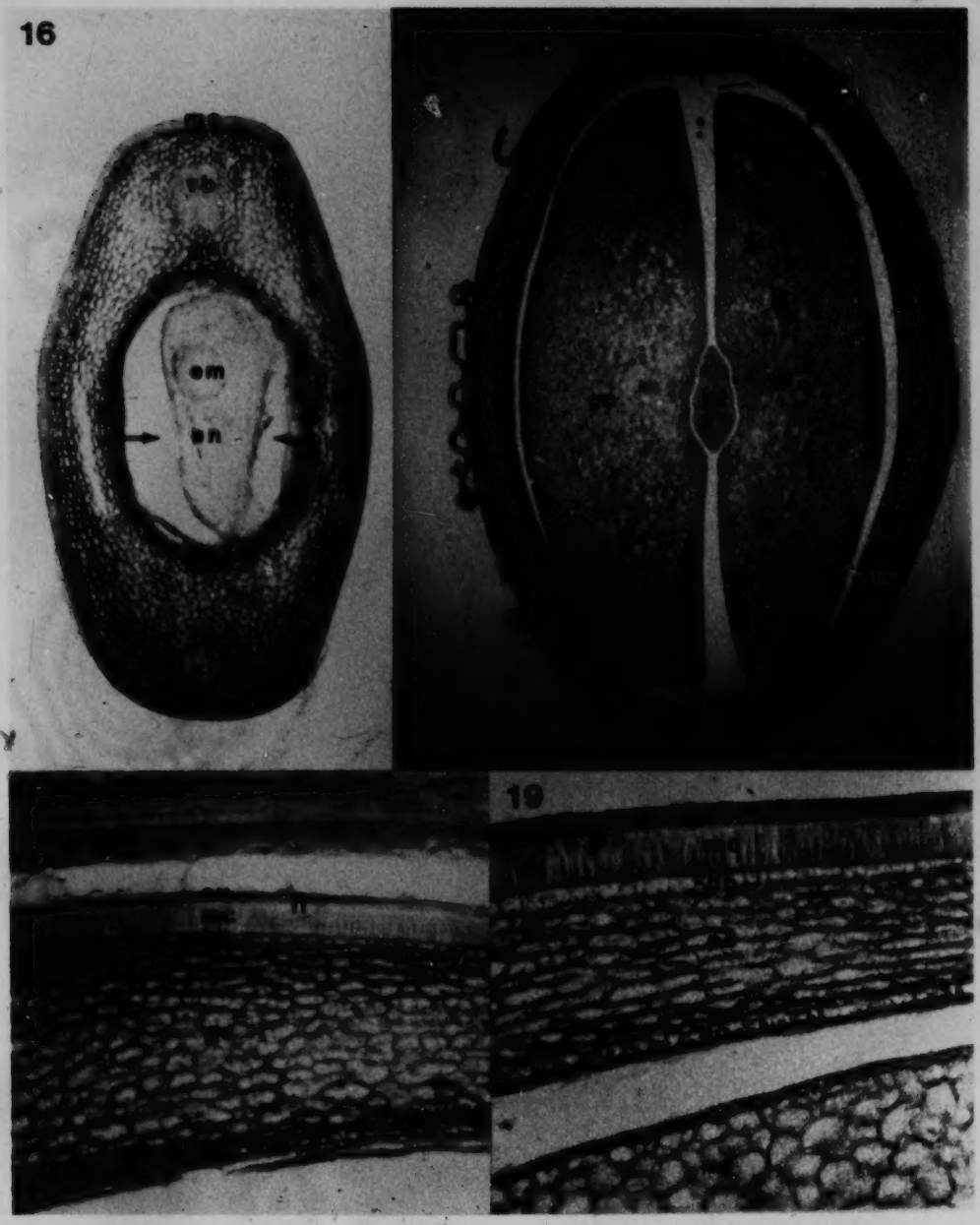
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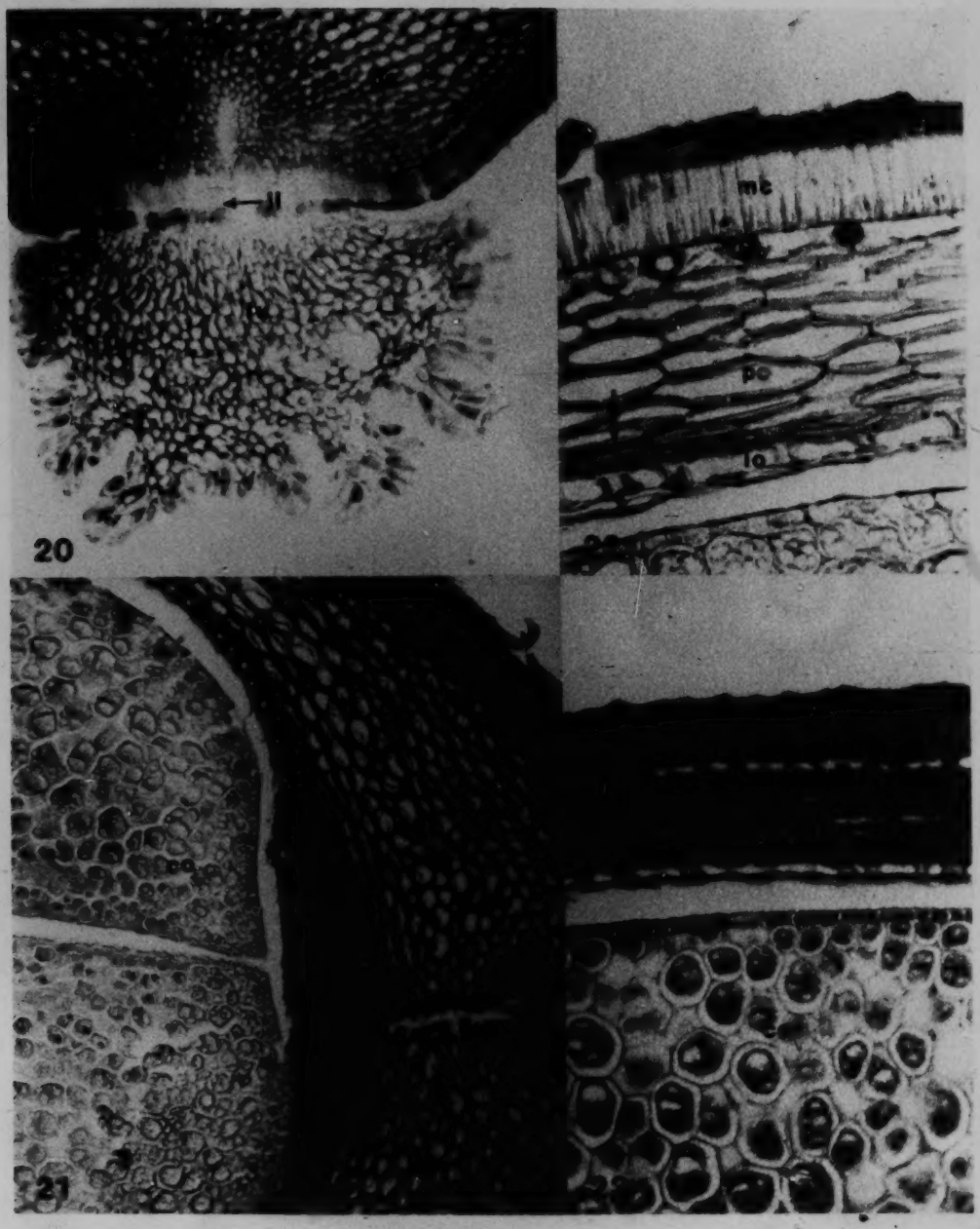
Figure 20. Longitudinal section of the seed coat showing the hilar region. The specimen was collected on August 15, 1985. The section was stained with PAS-Light green SF. The light line indicated by the arrow. X31.25.

Figure 21. Cross section of the seed coat showing some macrosclereid cells occurring in the inner osteosclereid cells. The specimen was collected on August 15, 1985. The section was stained with PAS - Light green SF. X78.12.

Figure 22. Cross section of the seed coat showing tangential wall thickening of parenchyma cells and crushed parenchyma cells at the innermost of parenchyma layer. The specimen was collected on August 22, 1985. The section was stained with Delafield's Hematoxylin - Orange G. The arrow shows crushed parenchyma cells. X78.12.

Figure 23. Cross section of the mature seed coat showing completely crushed parenchyma cells. The specimen was collected on September 12, 1985. The section was stained with Delafield's Hematoxylin - Orange G. X50.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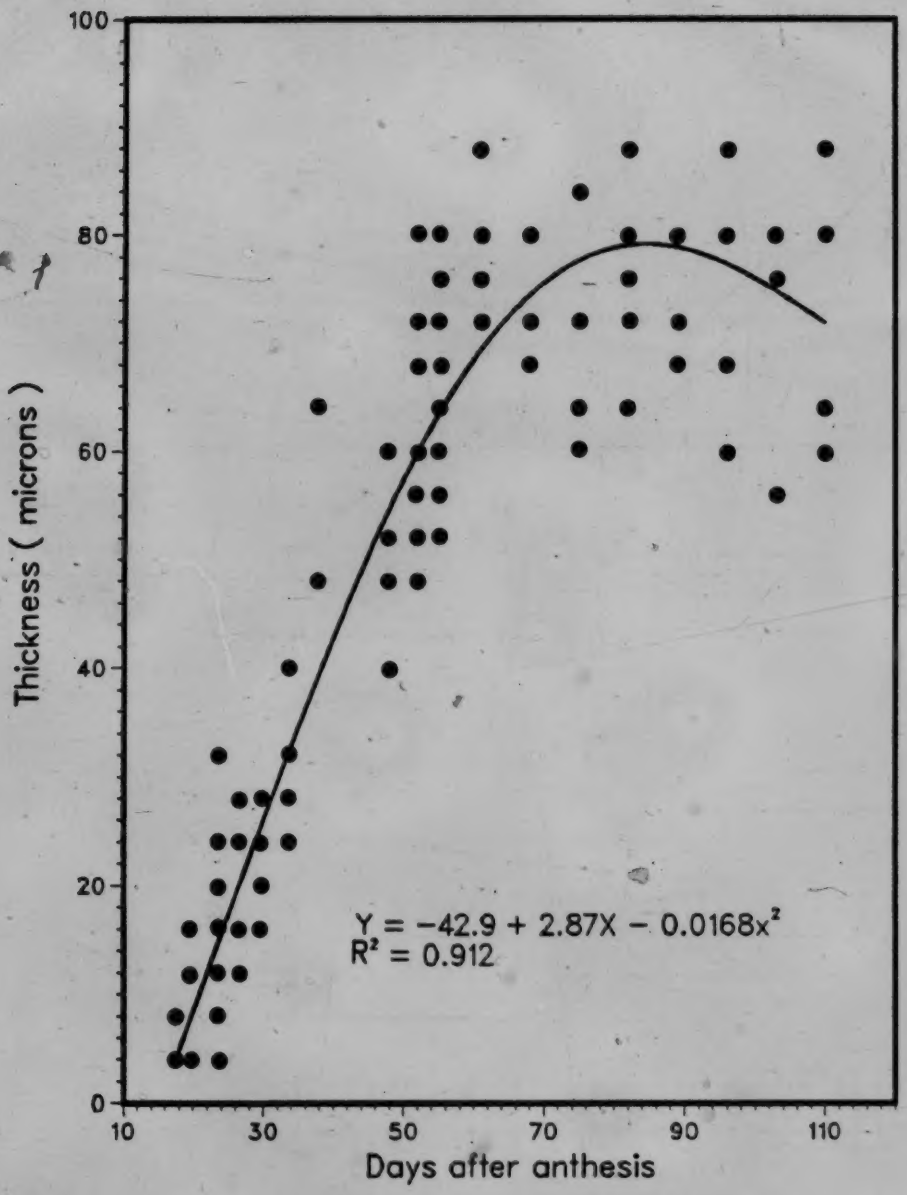

Figure 24. Thickness of macrosclereid plus cuticular layer during maturation. (n = 145, some dots have many observations).

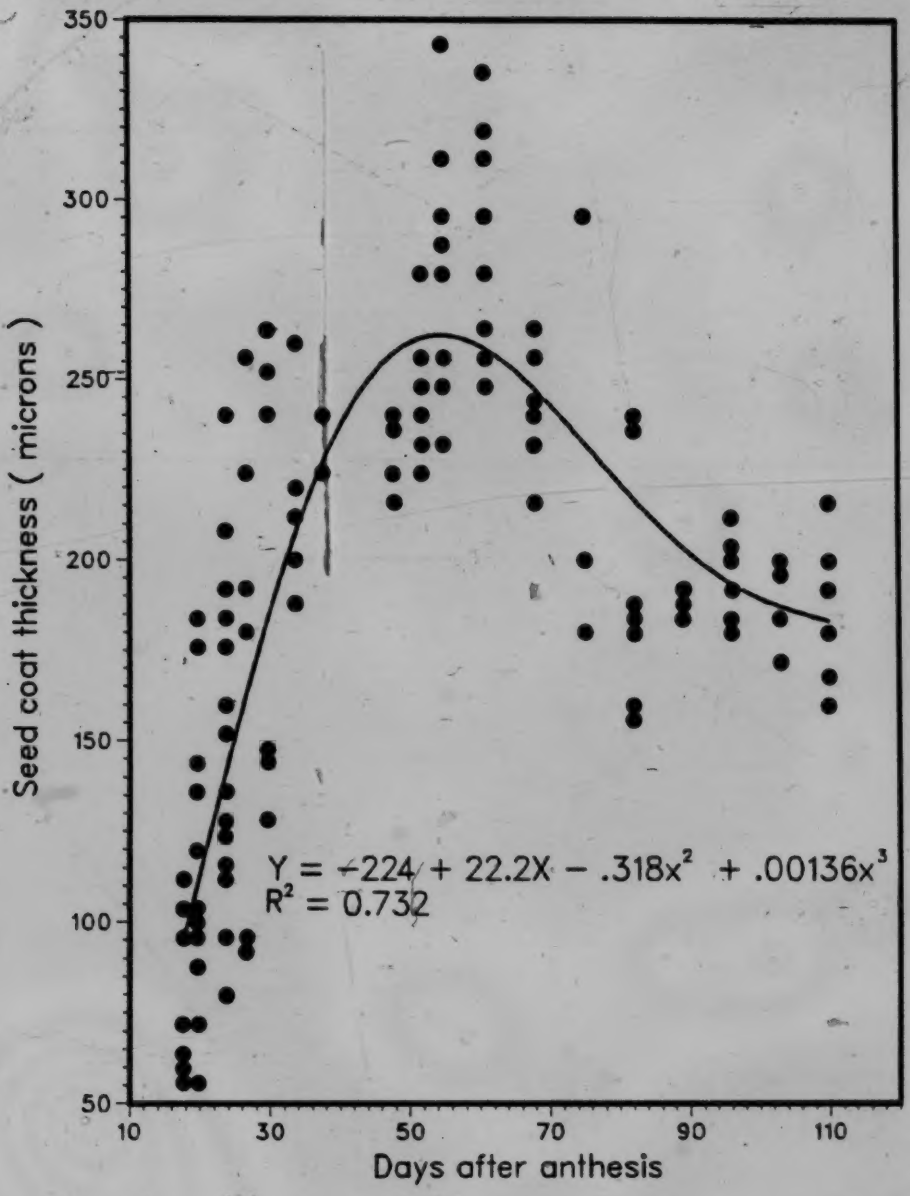

Figure 25. Thickness of seed coat during maturation. (n = 145, some dots have many observation).

3.3 Seed Coat Treatements

The results on germination response by using seed coat treatments are given in two sections as follows:

3.3.1 Effects of artificial treatments on germination

The effects of concentrated H_2SO_4 for 30 minutes and 95°C water for 3 minutes on seed coat surfaces are shown in Figure 26.

Seeds treated with concentrated H_2SO_4 for 15 and 30 minutes began to germinate on day 4, a day earlier than the others (Figure 27). Seeds subjected to hand scarification and concentrated H_2SO_4 for 15 and 30 minutes germinated almost completely two weeks after sowing while those treated with concentrated H_2SO_4 for 5 minutes, 95°C water for 1, 3 and 5 minutes increased germination gradually, and nearly completed it in 3 weeks. Control seeds and those treated with concentrated HCl for 15 minutes and 10% KOH for 24 hours germinated slowly. Only total germination of seeds treated with 10% KOH for 24 hours was lower than that of control. Analysis of variance for total germination showed significant differences among those treatments (Table 20). Multiple comparisons for mean total germination indicated that seeds subjected to 95°C water for 1 and 3 minutes had the highest germination of 95.5% and differred from those treated with concentrated H_2SO_4, hand scarification and 95°C water for 5 minutes (Table 22). Within 3 days, from day 4 to day 6 after sowing, seeds treated with hand scarification and concentrated H_2SO_4 for 15 and 30 minutes increased

Figure 26. Effects of some treatments on seed coat surface.
A : Control. B : 95°C water for 3 minutes.
C : Concentrated H_2SO_4 for 30 minutes.
D : Germinating seedlings treated with concentrated H_2SO_4 for 30 minutes.

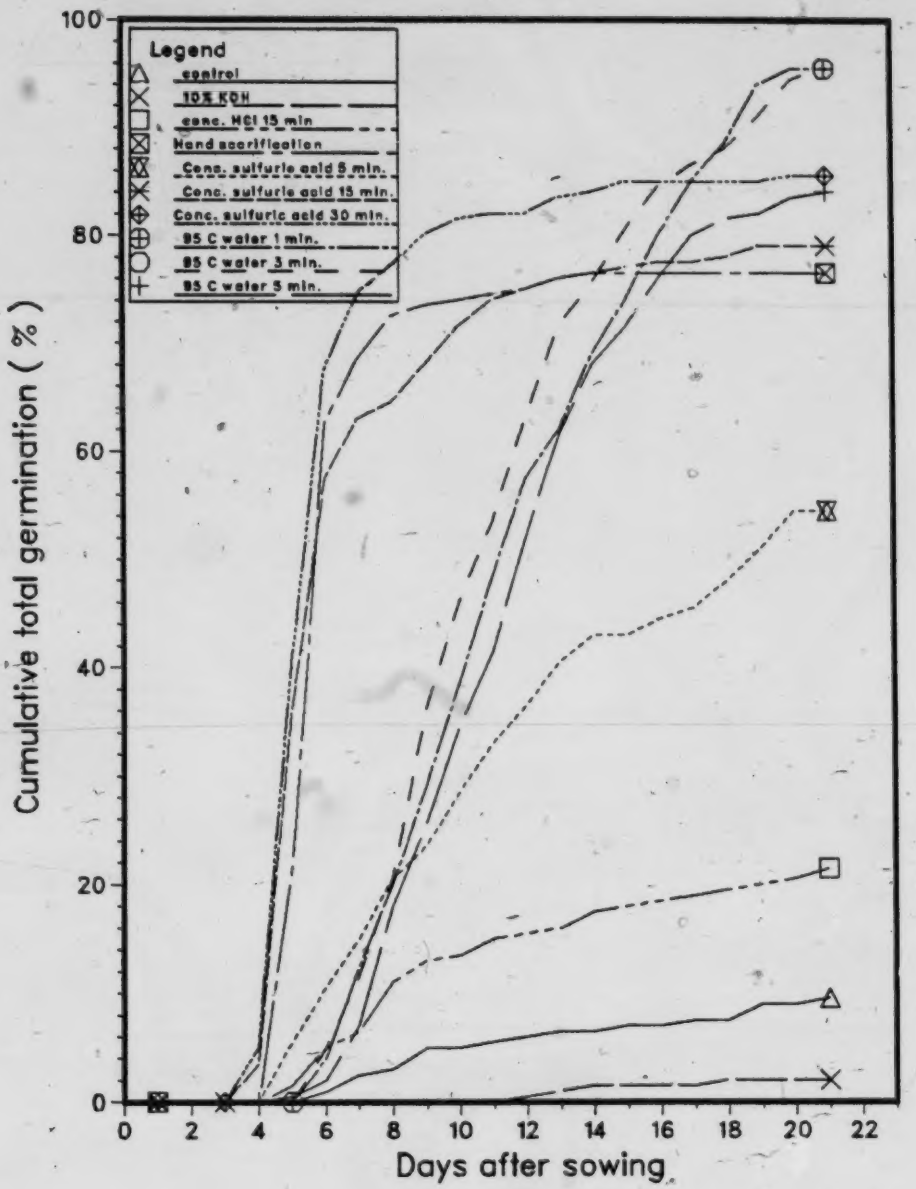

Figure 27. Percent cumulative germination of *A. auriculiformis* subjected to different seed coat treatments.

germination greately from 0-5% to 57-67% (Figure 27). Analysis of variance for germination rate showed significant differences among seed coat treatments (Table 21). Multiple comparisons for mean germination rates showed that seeds treated with concentrated H_2SO_4 for 15 and 30 minutes and hand scarification achieved their 50% of total germination around 5 to 6 days, while those treated with 95°C water and concentrated H_2SO_4 for 5 minutes needed around 10-11 days (Table 22). Seeds treated with 10% KOH for 24 hours showed the worst total germination (2%) and germination rate (14 days).

Table 20. ANOVA for total germination by seed coat treatments.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	9	47300.03	5255.56	196.4	.0000
Within groups	30	802.75	26.76		
Total	39	48102.78			

Table 21. ANOVA for germination rate by seed coat treatments.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	9	313.32	34.81	27.63	.0000
Within groups	30	37.80	1.26		
Total	39	351.12			

Table 22. Comparisons of mean germination and germination rates among seed coat treatments.

Treatment	Germination (%) Mean	S.D.	Germination rate rate(days) Mean	S.D.
Control	9.50 e	2.52	8.63 b	1.11
10% KOH (24 hrs.)	2.00 f	1.63	14.03 d	2.48
Conc. HCl (15 min.)	21.25 d	8.26	8.00 b	0.71
Hand scarification	76.50 b	7.72	5.40 a	0.12
Conc. H_2SO_4 (5 min.)	54.50 c	4.12	9.80 bc	1.76
Conc. H_2SO_4 (15 min.)	79.00 b	8.41	5.22 a	0.36
Conc. H_2SO_4 (30 min.)	85.50 b	4.12	5.08 a	0.19
95°C water (1 min.)	95.50 a	1.91	10.93 c	0.66
95°C water (3 min)	95.50 a	1.91	10.15 bc	0.46
95°C water (5 min.)	84.00 b	4.32	10.98 c	0.89

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

3.3.2 Effect on collection dates

From the results in section 3.3.1, the best treatments to overcome seed coat dormancy in the laboratory were 95°C water for 1 and 3 minutes because they gave the highest total germination and less mold development on the seed beds. Between these two treatments, 95°C water for 3 minutes was decided to be a treatment for this study because it enhanced the germination rate.

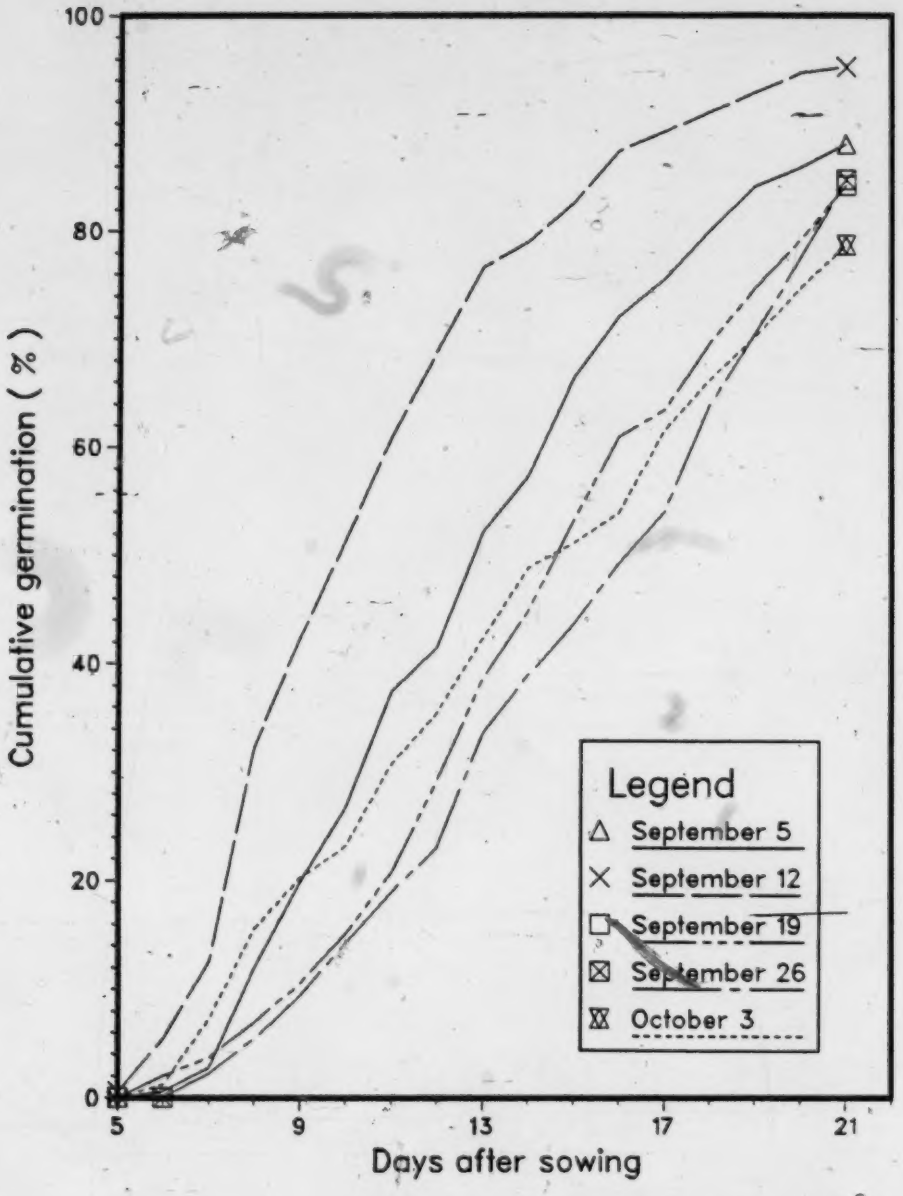

Figure 28. Percent cumulative germination of seeds of different collection dates treated with 95°C water for 3 minutes.

Seeds of all collections began to germinate on day 6 except those collected on September 12 which began on day 5 (Figure 28). Seeds of all collections did not complete their germination on day 21 after sowing, and a few other seeds still germinated during the week after. The regression models for total germination and germination rate were illustrated in Figure 29 and 30, respectively. Analysis of variance for total germination and germination rates showed significant differences among collection dates (Tables 23 and 24). Multiple comparisons for mean total germination and germination rates are shown in Table 25. Seeds collected on September 12 had the best germination rate; however, its mean total germination did not differ from seeds collected on September 5, 19 and 26 but differed from those collected on October 3.

Table 23. ANOVA for total germination of treated seeds.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	587.83	146.96	4.79	.0109
Within groups	15	460.36	30.69		
Total	19	1048.19			

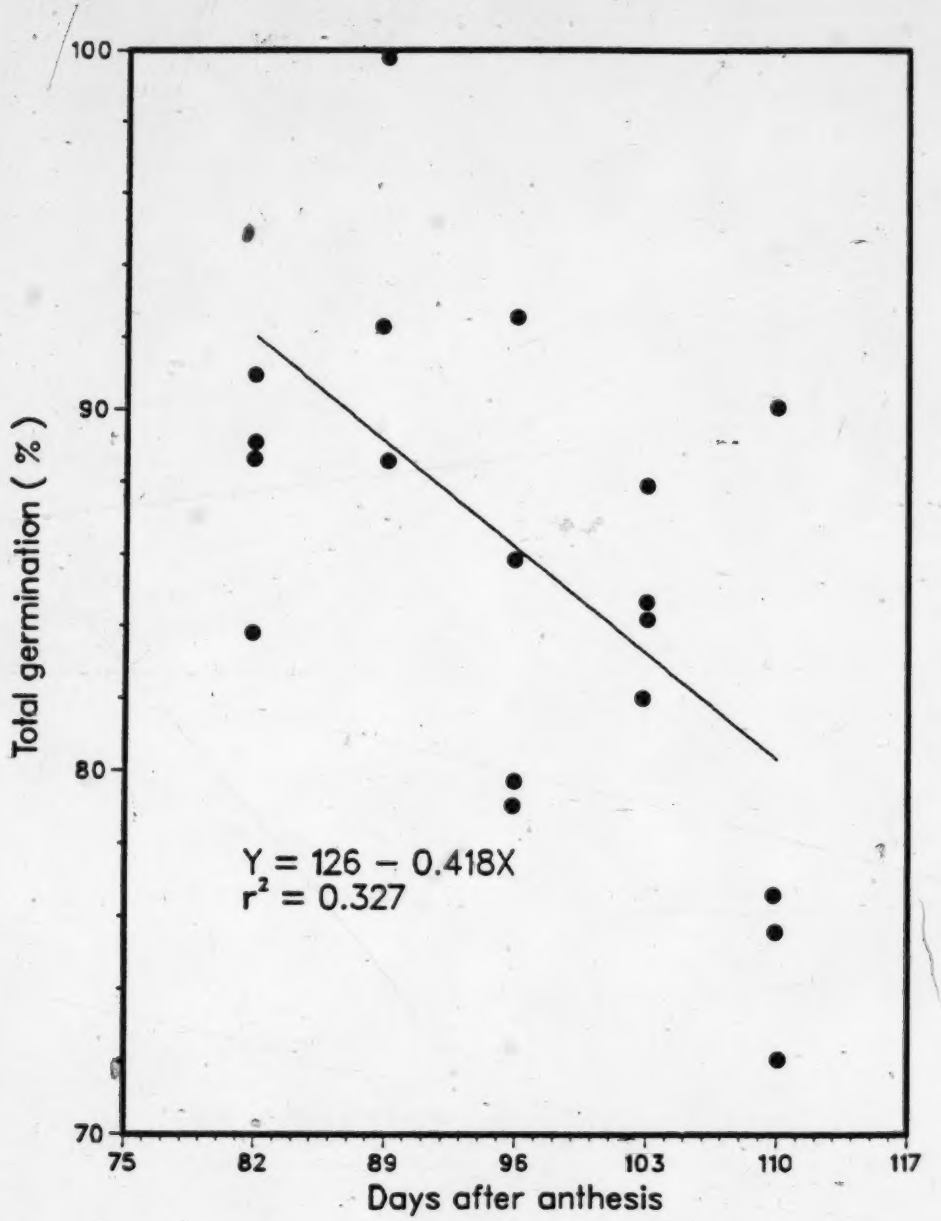

Figure 29. Changes in total germination of treated seeds two months after collection.

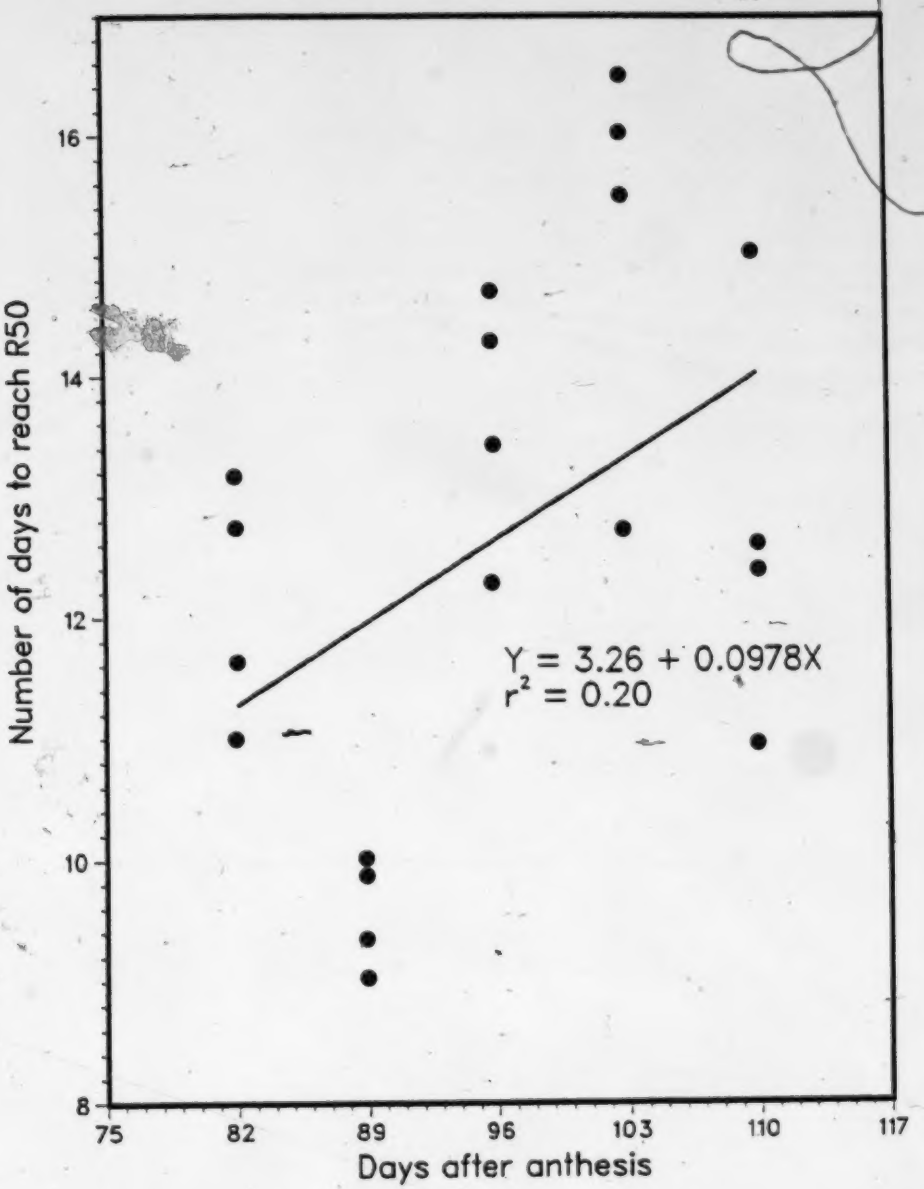

Figure 30. Changes in germination rate of treated seeds two months after collection.

Table 24. ANOVA for germination rate from treated seeds among collection dates.

Source	d.f.	S.S.	M.S.	F ratio	F prob.
Between groups	4	69.15	17.29	10.54	.0003
Within groups	15	24.54	1.64		
Total	19	93.69			

Table 25. Comparisons of mean germination and germination rates for treated seeds among collection dates.

Collection date	Germination (%) Mean		S.D.	Germination rate (days) Mean		S.D.
Sept. 5	88.04	ab	3.05	12.14	b	1.01
Sept. 12	95.18	a	5.79	9.54	a	0.46
Sept. 19	84.24	ab	6.42	13.64	bc	1.07
Sept. 26	84.68	ab	2.40	15.17	c	1.70
Oct. 3	78.66	b	7.98	12.75	b	1.70

(Comparisons followed by the same letter are not significantly different at the level $P \leq 0.05$).

4. DISCUSSION

4.1 Seed Maturation and Collection

Seed maturation and collection in *A. auriculiformis* have been discussed in considerable details in maturity indices, and germination and dormancy behavior in relation to climate conditions when seeds were collected.

4.1.1 Maturity indices

Both pericarp color and seed coat color were good indicators of maturity in *A. auriculiformis*. However, all mature seeds were completely black in color one week (September 5) before the pericarps became completely brown (September 12) (Figures 3 and 4). Funiculus color should not be used to define maturity because there is color mixing within the same funicle between green and yellow and between yellow and orange. Also the funiculus color changed quickly from green to orange. Some green and brown seeds already had orange funicles. Thus, this qualitative criterion could easily lead inexperienced seed collectors to erroneous determination of maturity.

The average moisture content of both fruit and seed of this species followed the expected pattern, as it decreased with time. Fruit moisture content varied greatly among collection dates varying much more than seed moisture content. Perhaps, the reason for this is that fruits experience changes in climate before the seeds do during the humid season. Thus, fruit moisture content appeared less

reliable than seed moisture content as a quantitative index of maturity. However, because of the gradual decrease in seed moisture content during maturation, seed moisture content alone appeared to be a less reliable index. Therefore, this method for determining maturity must be combined with other indices before specifying maturity.

Both seed fresh weight and seed dry weight indicated maturity consistently (Figure 7). Both seed weights can be divided into three phases during maturation. The first phase was the period of active growth characterized by a rapid increase in seed weight due to a large nutrient flow from the maternal tree into the seeds. At the maximal seed fresh weight, seed moisture content was about 39%. Secondly, it was followed by a desiccation period in which seed fresh weight dropped while dry weight increased only slightly. The last phase showed constant levels both in fresh and dry weights. However, the time to reach the constant levels differed between fresh and dry weights; seeds reached their maximal dry weight after September 5 (82 days after anthesis) while they attained their constant fresh weight by September 19. Similar results were shown by the study of Kermode and Bewley (1985) in *Ricinus communis* L. seed development and maturation.

Furthermore, morphological maturity as examined through X-ray radiographs showed that most seeds matured after September 5 (Table 5).

For these reasons, it can be concluded that seeds began ripening after September 5 (82 days after anthesis): seed

coat color was then black, seed moisture content was about 27%, seeds reached a maximal dry weight level of 17 mg by this time, and seed content from X-ray analysis indicated that the embryo was fully developed.

Nevertheless, local weather conditions also play a significant role in seed maturation. For example, the intensity and frequency of rainfalls during the rainy season in the Muak-Lek area in 1985 influenced seed maturation in *A. auriculiformis*. Contrary to the climatic diagram in Figure 2, the amount of precipitation in July, August and September of 1985 was 173.1, 58.3 and 233 mm, respectively (data from the Thai Danish Diary Farm). As Walter (1979) pointed out; a month with less than 100 mm of rain in the tropical climate is considered to be relatively dry. Therefore, the month of August 1985 was an unusually dry period in the rainy season. As a result, the seeds of *A. auriculiformis* showed a rapid decrease in fruit and seed moisture content from August 22 to September 5 (day 68 to 82 after anthesis) (Figure 6). Then, they increased in weight during the following two weeks, from September 5 to 19 when the intense rains started again. Therefore, it is possible that some seeds from August 22 to September 5 may have entered a stage of muturity earlier, and at least some mature seeds by September 5 could absorb some moisture from the surroundings.

4.1.2 Germination and dormancy

Judging by the behavior in seed germination and dormancy, it is clear that germination and dormancy are dependent on the maturation processes. Germination behavior can be distinguished by two phases: (i) germinability increased to a peak on September 12, with high seed moisture content from 32% to 23% and (ii) a decline in germinability followed, with the moisture content of seeds falling below 23% (Figure 8). Seed dormancy also showed two phases: (i) dormancy due to immaturity before September 12, and (ii) natural dormancy associated with loss of seed moisture content after September 12 (Figure 8). During the first phase of germination and dormancy behavior, the percentage of ungerminated seed was still high because some seeds were immature, less vigorous and not able to germinate, yet they were vigorous enough to be viable during the germination period. In addition, the drought in August may have forced some seeds to become dormant earlier than normal. On the other hand, seed maturation in *A. auriculiformis* showed a shift from germinability to the dormant stage by September 12.

Also, seed mortality increased from early to late collections (Figure 8). Harrington (1972) suggested that seeds stored on the plant after maturity have shorter longevity than do those which are collected early because they are subject to more severe environmental influences. Therefore, if *A. auriculiformis* seeds were not collected before September 5, they were subject to these environmental

conditions. The viability of these seeds may deteriorate due to high rainfall and high temperatures during the rainy season.

Judging by the germination rates at the time of collection, it is surprising that the highest total germination and the most rapid germination rate did not occur at the same collection time (Figures 8 and 9). It is possible that, after maturity, seeds collected on September 19 and 26 were subject to weather conditions, particularly rainfall, longer than seeds collected on September 12. Thus, some seeds which regained moisture may be capable of germinating faster due to reduced times for water absorption.

From the result of germinability two months after collection, the average germination percentages for all collections declined remarkably compared with the germinability at the time of collection (Figures 8 and 10). On the other hand, seed dormancy increased greatly. The early seed collections appeared to have a higher percentage of seed dormancy or a deeper seed dormancy than those collected later (Figure 10). This behavior may be the result of seeds losing their own moisture during the two months of storage when drying took place (Figure 31). As Mayer and Poljakoff-Mayer (1982) pointed out, if the seeds are exposed to different microclimatic conditions during maturation, they might be drying at different rates. In this case, when

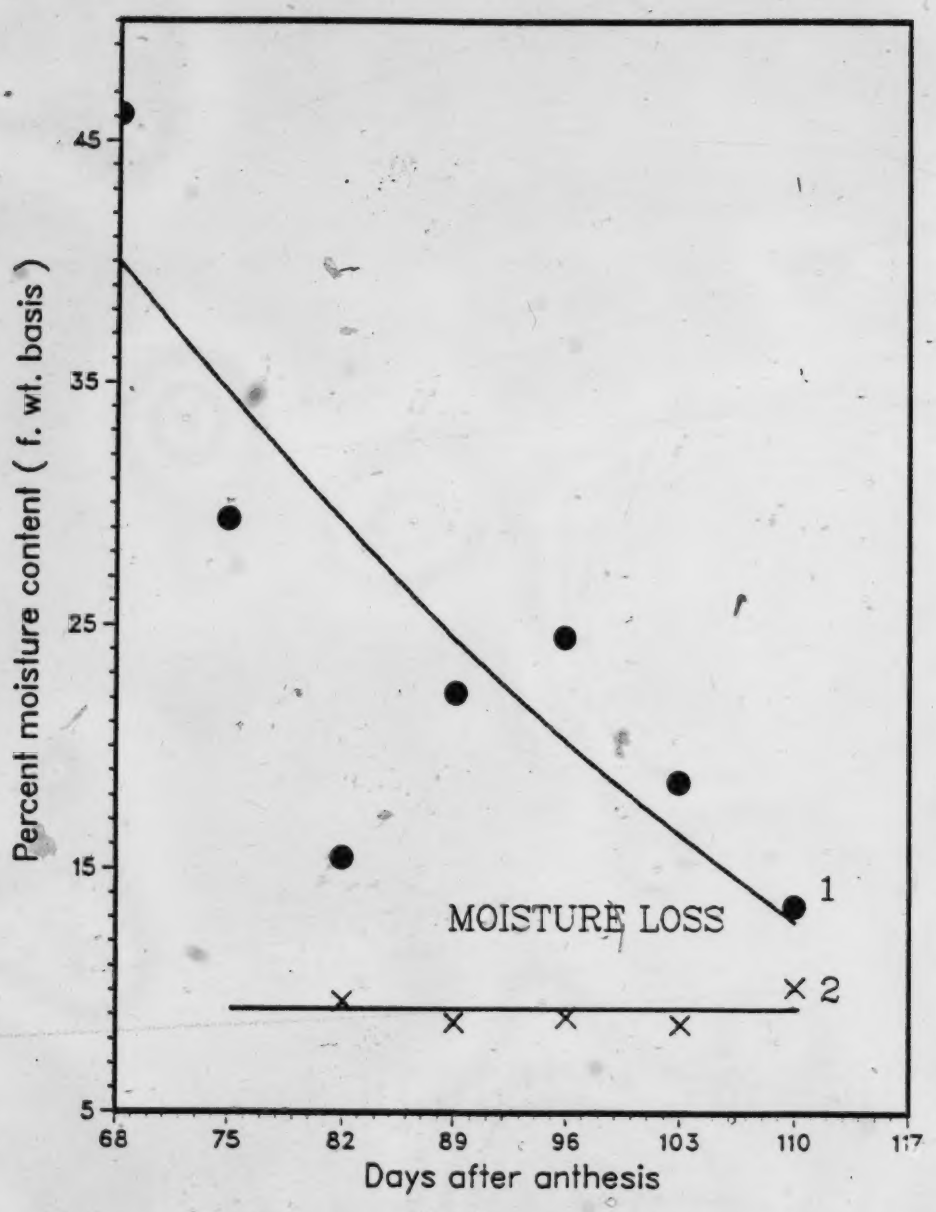

Figure 31. Changes in seed moisture content during two months of storage from the time of seed collections from September 5 to October 3.
(1): part of seed moisture content at the time of collection from Figure 6.
(2): seed moisture two months after collection.

comparing seeds drying inside the pods attached to the maternal tree with seeds outside the pods after harvesting, *A. auriculiformis* seeds harvested earlier had a high moisture content but lost their moisture content faster than did seeds which were left in the pods of the trees (Figure 31). Thus, with fast-drying, seeds of early collections may change seed properties drastically to produce deep dormancy. As a result, seeds of early collections needed more time (low germination rate) to recover their germinability than late collections (Figure 11).

Consequently, when considering the ungerminated seeds in Figure 10, the imposed dormancy has two patterns: (i) general dormancy imposed during the slow desiccation period of natural seed maturation due to hardening of the seed coats, and (ii) dormancy imposed as a result of the fast desiccation period after harvesting but before natural seed shedding took place (Figure 32). These two dormancy types work in opposite ways. In the former, dormancy tends to increase, while in the latter, it tends to decrease with late harvesting.

4.1.3 Ecological viewpoint

From an ecological point of view, the developmental period of *A. auriculiformis* seeds in this seed collection area in 1985 was influenced closely by climate. The trees began to flower in early May at the beginning of the rainy season. Thereafter, seeds continued to develop in the rainy

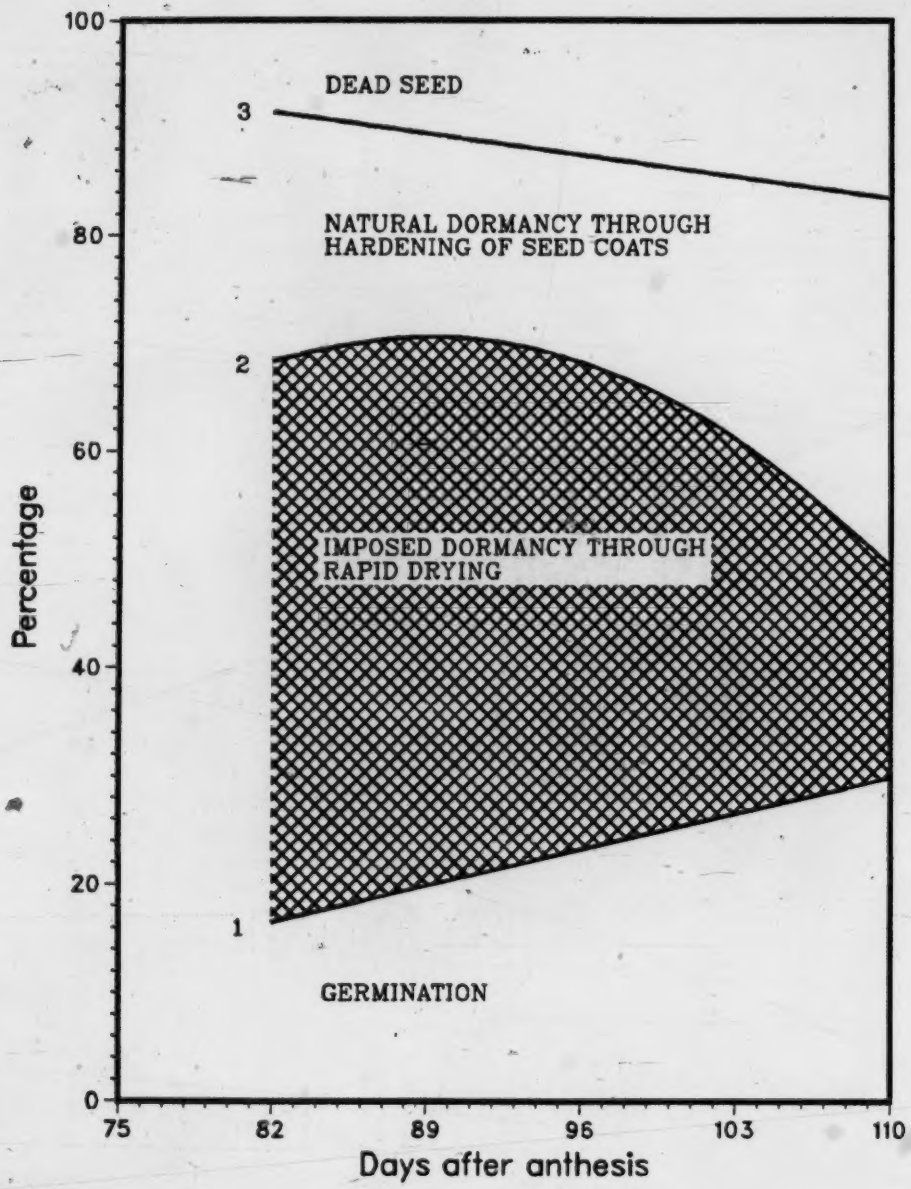

Figure 32. Natural and imposed dormancy development. Curve details are from Figure 8 (#2) and Figure 10 (#1 and 3).

season, and they were dispersed toward the latter part of the rainy season. The dispersal of *A. auriculiformis* seeds occurred from August 15, the middle of the rainy season, to October 6 towards the end of the rainy season. To succeed in the regeneration process, *A. auriculiformis* may adapt in germinability and seedling establishment. The seeds which matured earlier, on August 15 to 29, are capable of germinating and becoming established rapidly with the help of frequent and intense rainfall in the mid rainy season; while most seeds which matured later in the rainy season entered dormancy. Seeds which germinated late in the rainy season may not survive the following long dry season due to desiccation. On the other hand, some seeds have deeper dormancy due to being dried further. However, during the dry season, some seeds may experience seed coat damage. Therefore, when the next rainy season comes, these seeds, depending on how damaged they are, are permeable to water and gas penetration which enhance their germination; otherwise, they die in the rainy season. According to Preece (1971), only 2% of the seedfall of *A. aneura* Benth. was capable of germination the first time rains came and the temperature was between 20° and 30°C, and the remainder of the seeds would await the breakdown in hardseededness which occurred after some time.

4.2 Seed Coat Structure and Development

The seed coat structure of *A. auriculiformis* in this study was different than described previously (Corner 1951 and Dnyansagar 1958). As in Corner's work, the seed coat of *A. auriculiformis* does not have the layer of inner osteosclereid cells. Also, Dnyansagar's work showed that the seed coat of *A. auriculiformis* is composed of cuticle, macrosclereids and parenchyma. It would appear that Dnyansagar had not studied the mature seeds, but examined young developing seeds, as he stated that the specimens were very hard, and sections on slides were lost due to hardness and difficulty with which they adhered to the slides. Similarly, some problems occurred with the sections such as the separation of the cuticle from the seed coat, and sections which were brittle and curly.

Robbertse's work (1974, cited by Cavanagh 1980), like that of Corner, showed that the seed coat structures of *A. grandicornuta* from Africa is composed of all structures except the inner osteosclereid layer. If such is the case, the difference in seed coat features among Australian and African *Acacia* species may be useful in taxonomic classification. Also Khudairi (1956) showed that the seed coat of *Prosopis stephaniana*, belonging to the genus *Prosopis* of Mimosoideae, is composed of a thick cuticle, macrosclereid layer, several layer of parenchyma and a single inner layer of thin-walled cells. Thus, the difference in the hypodermal osteosclereid layer and the inner layer of seed coat structures is helpful for further

taxonomic investigations within this subfamily.

In the present study, an attempt has been made to follow the close sequence of seed coat development as it relates to seed maturation. Investigation of the developmental anatomy of *A. auriculiformis* seed coat ascribed differences in seed coat structures and morphometries. The results in this present study suggest that the morphological development of the seed coat is relative to seed maturity. Seed maturity in this species is shown by the completely compressed parenchyma and the constant thicknesses of the macrosclereid plus cuticular layer. These morphological maturity indices correspond with the previous maturity indexes beginning with September 5. The thickness of the macrosclereid plus cuticular layer appeared to shrink by September 5 (82 days after anthesis). However, it seems likely that the hardness and impermeability of the seed coat are not the results of this shrinkage as seeds are able to absorb water in September and seeds can germinate at the time of collection.

4.3 Seed Coat Treatments and Germination

In this viewpoint, the two discussions have been stated on the effects of seed coat treatments on the germination responses. These, therfore, will be considered in the following sections.

4.3.1 Effects of various artificial treatments

There were great differences in the germination responses in *A. auriculiformis* seeds when they were subjected to various seed coat treatments. When comparing these treatments with control, they can be divided into four groups:

1. Treatment which inhibited the germinability and the germination rate: 10% KOH for 24 hours.
2. Treatment which improved the germinability less than 50%, but did not improve the germination rate: concentrated H_2SO_4 for 5 minutes and concentrated HCl for 15 minutes.
3. Treatment which promoted the germinability more than 65%, but did not promote the germination rate: 95°C water for 1 to 5 minutes.
4. Treatment which enhanced both the germinability more than 65% and the germination rate: concentrated H_2SO_4 for 15 and 30 minutes and hand scarification.

In fact, concentrated H_2SO_4, 95°C water and hand scarification overcame the dormancy in *A. auriculiformis*. When comparing treatments in group 3 and 4 above, we see that, even though treatments in group 4 can improve both the total germination and the germination rate, they still had less total germination by 10% to 19% than those in group 3 (except for 95°C water for 5 min). Moreover, mold development was a serious problem for seeds treated with

concentrated H_2SO_4 and hand scarification (group 4) while those in group 3 had less this problem. It is possible that the heat from hot water inhibited the growth of seed-borne pathogens (Maude 1972). Therefore, treatments of group 3 are preferred to enhance the germinability in the laboratory.

However, seeds which germinate early are able to exploit natural resources first such as light, water, nutrients; therefore, they can occupy their own teritories and have a better chance for survival in nature (Harper 1977). Furthermore, not only seed-borne pathogens but also soil-borne pathogens play a role in destroying seeds. The longer seeds remain unable to germinate, the better chance for mold to attack seeds.

In general, options may be chosen in the nursery procedures to enhance the germinability of *A. auriculiformis* seeds. The first way is to treat seeds with concentrated H_2SO_4 for 15 to 30 minutes. In this case, uniform seedlings can be obtained. Also when in a rush, this treatment shortens the time of seedling growth in the nursery beds. On the other hand, with a large scale program, many workers are needed to transplant young seedlings into the containers because many seeds germinate at the same time.

Secondly, in general, the pretreatment using 95°C water for 1-3 minutes should be preferred in practice in Thailand because it is easy to work with, and the cost is low compared to the concentrated H_2SO_4 treatment. With hot water, non-uniform seedlings will be obtained from the gradually germinating seeds. Therefore, only a few workers

are needed for transplanting. Seedlings from seeds which germinate on the same day, are grouped together in the nursery. These seedlings within the same block in the nursery show similar growth rates. As a result, grading of seedlings within the block take less time.

Finally, manual scarification may be used to enhance the germinability, but time and manpower are the main problems with a large scale operation. However, the invention of a machine for mechanical scarification could aid nursery work.

It is clear from the literatures on leguminous seeds that water is absorbed through slits on the seed coat surfaces (Brant et al. 1971; Halloran and Collin 1974; Liu et al. 1981) and through hilar fissures (Burns 1959) after being scarified. In this study, the seed coat surface of *A. auriculiformis* showed strongly damaged pleurograms when seeds were soaked in concentrated H_2SO_4 and hot water (Figure 26). Therefore, it is possible that the pleurograms and the pleurogram areas are the weakest sites at which water penetrates into the seed.

Hyde (1954) stated that the hilum in Papilionaceae (in family Leguminosae) functions as a hygroscopic valve. It closes when the relative humidity of the atmosphere around the seed is higher than that in equilibrium with seed moisture content, and opens in the reverse situation. Before his death, he had drafted a paper discussing how the pleurograms (rings) of *Albizia* seeds functioned as a valve (Gunn 1981). Therefore, it is still doubtful how the

pleurograms function in mimosoid seeds.

However, when considering the role of seed-soil interface, the pleurograms probably play an important role in water entry into the seeds. As the shape of *A. auriculiformis* seeds is flattened, the zone of contact between seed surface and soil is on the flat side if the substrata are flat as well (Figure 26). The pleurograms are located on the flat sides of the seeds. Therefore, one of the pleurogram and pleurogram area will face the soil while the hilum is elevated slightly above the soil surface. When seeds are moved or seed coats are damaged with the dispersal agents or natural phenomena, the seed coat on the flat sides, including the pleurograms and the pleurogram areas, propably will be destroyed first. Therefore, the water absorption of the seed coats from the surroundings should take place on the roughly damaged seed coats rather than the smoothly intact seed coats on the same seed.

4.3.2 Effect on collection dates

Undoubtedly, the treatment with 95°C water for 3 minutes enhanced seed germination without damaging non-dormant seeds. Two months after collection, the percentage of total germination of treated seeds was nearly equal to the percentage of germinated plus ungerminated seeds without pretreatment (Figures 10 and 33). In addition, these treated seeds of all collections showed similarity in germination rates; germination increased gradually (Figure 28). As a result, it would appear that seed maturation,

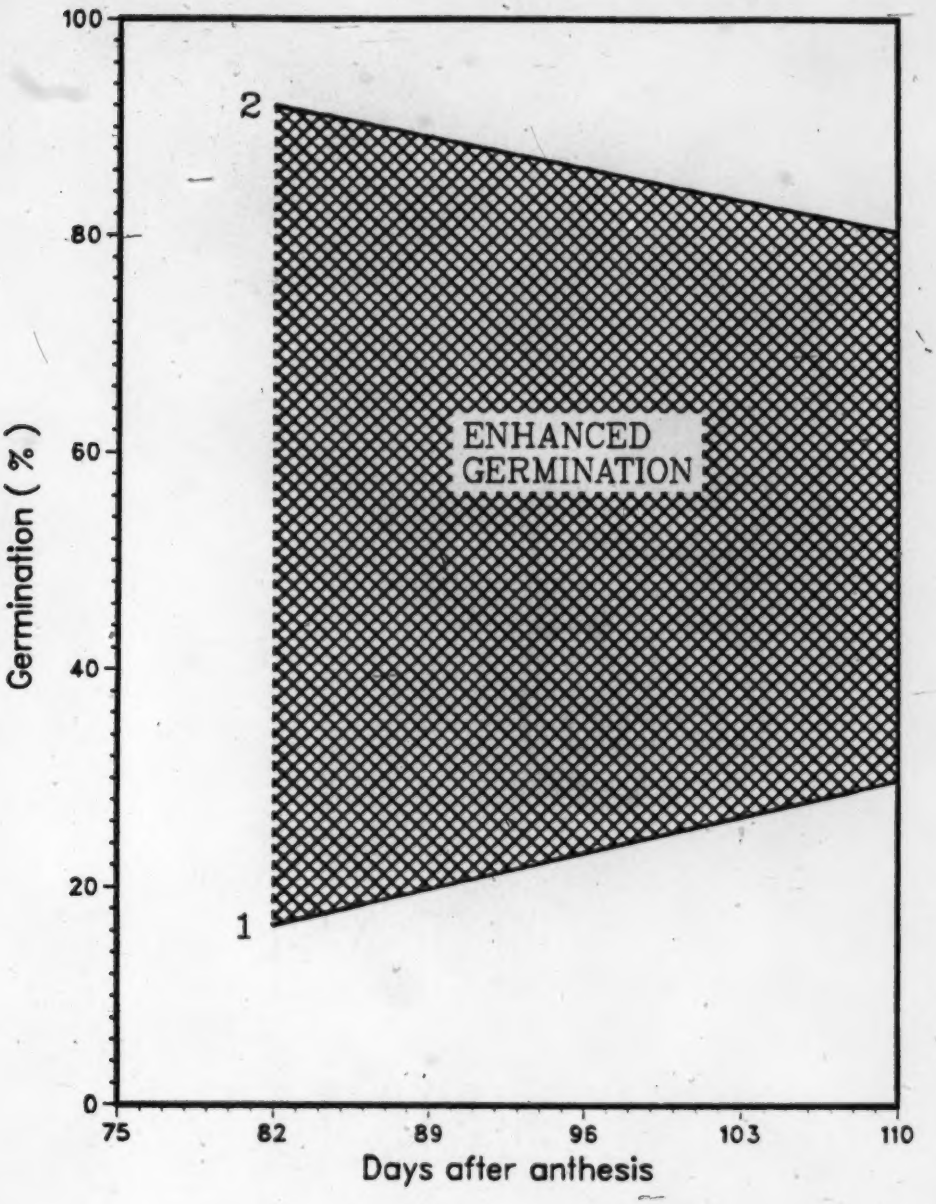

Figure 33. Enhanced germination of seeds treated with 95°C water two months after collection.
(1): Germination of untreated seeds from Fig. 10.
(2): Germination of treated seeds from Fig. 29.

rather than seed pretreatment, still influenced germination behavior even two months after collection. In other words, seeds tended to decreased in total germination (Figures 29 and 33) and germination rates (Figure 30) with increasingly later collections.

On the whole, seed collection procedures in *A. auriculiformis* have to be considered in the germination behavior before or after seed storage depending upon their specific purposes. When one wants to produce seedlings after collection, one should germinate seeds immediately and must not dry seeds after collection. On the other hand, when considering the seedling productions in the nursery for plantations, the collected seeds have to be stored about 2 to 5 months before sowing. With regard to seed viability, germination and germination rate of the treated seeds two months after collection, stored seeds should be collected as early as possible from September 5 to 12 (82-89 days after anthesis) depending on local weather. Later collections resulted in less seed viability due to a gradually increasing seed mortality. Late collections also gave less seeds as seeds were released gradually. Collections may be carried out before September 5, in order to extend the collection season and to avoid the weathering damage to the seed crop (Willan 1985). However, more information is needed on how to dry and store immature seeds without injury.

5. CONCLUSIONS

From the study of seed maturation, seed coat development and seed coat treatments in *A. auriculiformis* seeds, the following conclusions were drawn:

1. Seed maturity can be defined by various indices. They are pericarp and seed coat color, fruit and seed moisture content, seed dry weight, X-ray technique, seed coat structure and seed coat morphometries. Such maturity indexes indicated that seeds were mature after September 5 in 1985 at Muak-Lek.
2. Brown, opening pods and black seed coats were good as qualitative maturity indexes.
3. Fruit and seed moisture contents were reduced steadily with time from a high of approximately 70-85% 30 days after anthesis to 14% by 110 days after anthesis. In addition, fruit and seed moisture contents were about 35% and 27%, respectively, at maximum dry weight.
4. At maturity, seed reached maximum dry weight on September 5 with an average of 17 mg while seeds were drying further. Constant seed fresh weight was obtained later (on September 19) with an average of 20-21 mg.
5. Seed maturity indicated by X-ray radiographs showed that most of the seeds were mature after September 5 with the percent mature seed reaching approximately 90%.
6. Germination behavior of seeds was also affected by seed collection time. When seeds were collected, total germination still was high ranging from 50% to 77% with the peak occurring on September 12. However, the highest

germination rate was obtained 1-2 weeks after peak total germination. Two months after collection, total germination and germination rate decreased sharply.

7. Two types of seed dormancy was shown by (i) natural dormancy resulting from slow desiccation during natural seed maturation and (ii) imposed dormancy resulting from rapid desiccation after collection but before natural seed shedding took place.
8. The structure of immature seed coats is composed of a macrosclereid layer and parenchymatous layers while that of mature seed coats is composed of a thick cuticle, a macrosclereid layer, a hypodermal osteosclereid layer, parenchymatous layers and an inner osteosclereid layer.
9. Mature seeds had completely compressed parenchyma cells, a constant thickness of seed coat on the flat sides and a constant thickness of macrosclereid plus cuticular layer.
10. Treatments with 95°C water for 1 and 3 minutes led to a total germination to 95.5% while treatments with concentrated H_2SO_4 for 15 and 30 minutes and hand scarification led to 76.5-85.5% germination.
11. Within two months after collection, seed maturation still influenced seed germination.

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APPENDIX 1

Fixation, Dehydration and Infiltration for the paraffin method.

1. Early developing seed.

Fixed in FAA.....1-2 days.
Dehydrated in TBA series
-10% TBA.....3 hrs.
-20% TBA.....3 hrs.
-30% TBA.....3 hrs.
-55% TBA.....3 hrs.
-75% TBA.....3 hrs.
-100% TBA.....3 hrs.
-repeat 100% TBA.....overnight
Infriltrated
-100% TBA:paraffin oil,1:1..6 hrs.
-pure paraffin.....overnight
-repeat paraffin, twice.....6 hrs each

note: FAA = Formalin-Acetic acid-Alcohol
TBA = Tertialy Butyl Alcohol

2. Early mature and mature seed.

Seeds were cut in half to expose the interior surface.
Fixed in FAA.....2 weeks or more
(FAA was changed each 2-3 days)
Dehydrated in TBA series
-10% TBA.....2 days.
-20% TBA.....2 days.
-35% TBA.....2 days.
-55% TBA.....2 days.
-75% TBA.....2 days.
-100% TBA, twice.....2 days each
(TBA was changed every day)
Infriltrated
-100% TBA:paraffin oil,1:1..2 days.
-pure paraffin.....1 day.
-repeat paraffin, twice.....2 days each.

APPENDIX 2

Example for calculation the thickness with an ocular micrometer.

100 ocular micrometer units correspond to 0.4 mm of the scale 0.5 mm of the stage micrometer for 40X magnification.

Thus 1 ocular unit corresponds to .004 mm or 4 μm.

The seed coat is 30 ocular units thick.

Thus the thickness of the seed coat is 120 μm.